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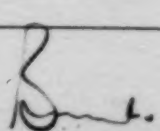
19th NOVEMBER 1984.	

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HUMAN GALLBLADDER MOTILITY EVALUATED BY ULTRASONOGRAPHY

by

PETER J. CANT

(C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

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SUPERVISOR (Dr. G.W. Scott)

.....
Dr. O.G. Thurston

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Dr. A.S. Clanachan

Dated November 19, 1984

Abstract

This research project evaluates the use of real time grey scale ultrasound in the investigation of human gallbladder motility.

The accuracy of the available methods of measuring gallbladder volume was assessed in human cadavers and a dog model, where the true volume of the gallbladder was known. It was found that ultrasonographic estimation of the volume was unreliable, but that measurement of the changing longitudinal area of a gallbladder as depicted by ultrasound was representative of its changing volume. A rapid, accurate estimation of changes in gallbladder volume can be made by computer digitization of the longitudinal cross-sectional areas recorded on film.

With these limitations in mind, a human gallbladder function study was developed. Normal male volunteers served as the study population. Parameters measured were the maximum area (A max), which is representative of the fasting gallbladder size; the rate constants of emptying and the percentage emptied in 30 mins (%E30), in response to a standard liquid meal. The latter two parameters indicate the sensitivity and contractility of the gallbladder and the resistance to the outflow of the bile. Analysis of the data showed broad normal variations in all these parameters, both between individuals and even within the same individuals. This restricts the use of the data for direct quantitative comparison.

A feature noted in the function study was the apparent increase in gallbladder volume following a test meal. This is analogous to Boyden's two minute pause and probably represents a pyloro - cholecystic reflex.

A prospective study was carried out on the effect of oral indomethacin, an inhibitor of prostaglandin synthesis, on gallbladder function in male volunteers.

Paired analysis of the data showed a significant decrease in A max in response to a four day oral course of the drug, and a tendency to decrease the rapid phase of emptying.

A single dose of indomethacin administered immediately before a test by suppository had no effect on the measured parameters.

Indomethacin influences gallbladder function in cholecystitis and the present study indicates that prostaglandins may be involved in normal function as well.

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In particular

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INTRODUCTION

That gallstones constitute a major health hazard is irrefutable, and as part of the continuing search into the factors responsible for this ubiquitous problem this project is directed.

PERSPECTIVES ON GALLSTONES

The aetiology of cholesterol gallstones remains nebulous despite some 200 years of investigation.[1,2] Opinion has swung from local to general causes, through infection, desquamation, supersaturation and motility.[3,4,5]

There are five stages to symptomatic cholesterol gallstone disease.[6] Firstly, a genetic or metabolic propensity to form supersaturated bile, which may not necessarily be manifest. Secondly, the chemical stage when bile becomes supersaturated with cholesterol. Next, the physical stage where the supersaturated bile is nucleated; growth of crystals[7] and agglomeration in macroscopic stones constitutes the fourth stage. Finally the stones may then cause symptoms by inducing cholecystitis[8] or blocking some part of the extra-hepatic biliary tree.

Lithogenicity

Although it can be stated that gallstones do not form in the absence of supersaturated bile, it is also true that many "normal" non stone formers do at times have lithogenic bile.[9]

Cholesterol remains in solution in the bile due to the formation of micelles with bile salts and phospholipids. Admirand and Small[10] demonstrated that the solubility of cholesterol could be described by the relative molar concentrations of these three components on

triangular coordinates. The lithogenic index derived[11] enabled comparison of gallstone formers with controls and showed a fairly clear separation. Furthermore, the hepatic and gallbladder bile from stone formers was saturated indicating that the liver was the source of lithogenic bile.[12] Supersaturation may occur by increased secretion of cholesterol or decreased secretion of bile acids. Phospholipids have an uncertain role at this time.

Increased Secretion of Cholesterol

High calorie diets, especially in the obese, lead to increased activity of hydroxymethylglutaryl Co A reductase, the rate limiting enzyme in cholesterol synthesis.[3] The result is increased secretion of cholesterol into the bile.[13] The effect of diets rich in cholesterol is unclear, but clofibrate increases cholesterol secretion.[5] A genetic predisposition to gallstone formation in certain racial groups has also been linked to biliary cholesterol.[12]

Decreased secretion of Bile Salts

Several specific examples of this are apparent. T tube biliary fistula with external drainage depletes bile salts in excess of production and leads to bile supersaturated with cholesterol.[14] Impaired ileal reabsorption due to resection or diseases such as Crohns and cystic fibrosis can lead to similar effects.[3]

However in the majority of stone formers these specific situations do not apply, and bile supersaturation is the result of subtle changes in the enterohepatic circulation.

The Enterohepatic Circulation

The bile salts expelled from the gallbladder after a meal are 98%

reabsorbed and returned to the liver for resecretion into the canaliculae.[14] During fasting, secreted bile is sequestered in the gallbladder and bile salt return to the liver decreases,[15] increasing lithogenicity. Thus normal subjects may intermittently secrete supersaturated bile.[16]

There is a feedback mechanism by which the concentration of bile salts in the portal circulation regulates the de novo synthesis of bile salts by liver cells, but details of whether it is rate dependant and how rapidly it responds are unclear. Secretion of cholesterol and lecithin is linked to total secretion of bile salts, but lag slightly so that the relative amounts are highest when total secretion of bile salts is lowest.[3]

This process may be accentuated should there be rapid discharge from the gallbladder or shortened bowel transit,[17] which is supported by the observation that many stone formers have small bile salt pools.[18] Removal of the gallbladder in some of these improves biliary lipid solubility.[19,20,13] Several studies[21,22,23] have shown that there is abnormally rapid emptying of the gallbladder in stone formers.

The rate of gallbladder emptying correlates negatively with the size of the primary bile acid pool, but not with the deoxycholate pool.[17] This is probably not due to inhibition of the hepatic synthesis, but rather due to a higher fractional turnover associated with rapid gallbladder emptying.[24]

Small bowel transit time correlates positively with the secondary, but not with the primary bile acid pool. The mechanism in this case may be loss of primary acids into the colon and consequent dehydroxylation to secondary acids.[17] There may also be an associated decrease in the

total bile acid pool and consequent increase in cholesterol saturation.

An alternative theory suggests that the liver is oversensitive to bile acid feedback, and is not compensating for the fractional turnover, leading to a smaller bile acid pool.[12,18]

Dietary Fibre

In gallstone patients, additional dietary fibre reduces biliary cholesterol concentration and decreases the proportion of deoxycholate in the bile salt pool.[25] Deoxycholate suppresses hepatic synthesis of chenodeoxycholate, which is an important determinant of biliary lipid solubility, and fibre may thereby reduce biliary lithogenicity.[26]

The cause of this change is uncertain, but increased colonic motility due to the fibre may decrease absorption of deoxycholate, or its synthesis by gut bacteria.[27] It is tempting to implicate this mechanism in the low incidence of gallstone disease seen in some third world countries.[1]

Precipitation of Microcrystals and Growth of Stones

Supersaturation with cholesterol does not invariably lead to gallstone formation. Nucleation must occur, either spontaneously (homogenous nucleation) in the presence of a highly supersaturated solution or by heterogenous nucleation in the presence of a foreign nucleus.[6]

Since heterogenous nucleation occurs in less saturated solutions, this mechanism is more likely. Possible nuclei include mucus secreted by the gallbladder,[28] desquamated cells, bacteria and other chemical salts. In addition, certain nucleating and anti-nucleating factors may be present,[29] together with local permissive or prohibitive conditions.[30]

The growth stage is reached once crystals have exceeded a critical size where dissolution is exceeded by crystalisation. Factors such as stasis[31,32] and fluid transport can facilitate or retard growth and agglomeration to macroscopic stones.

PERSPECTIVES ON PROSTAGLANDINS

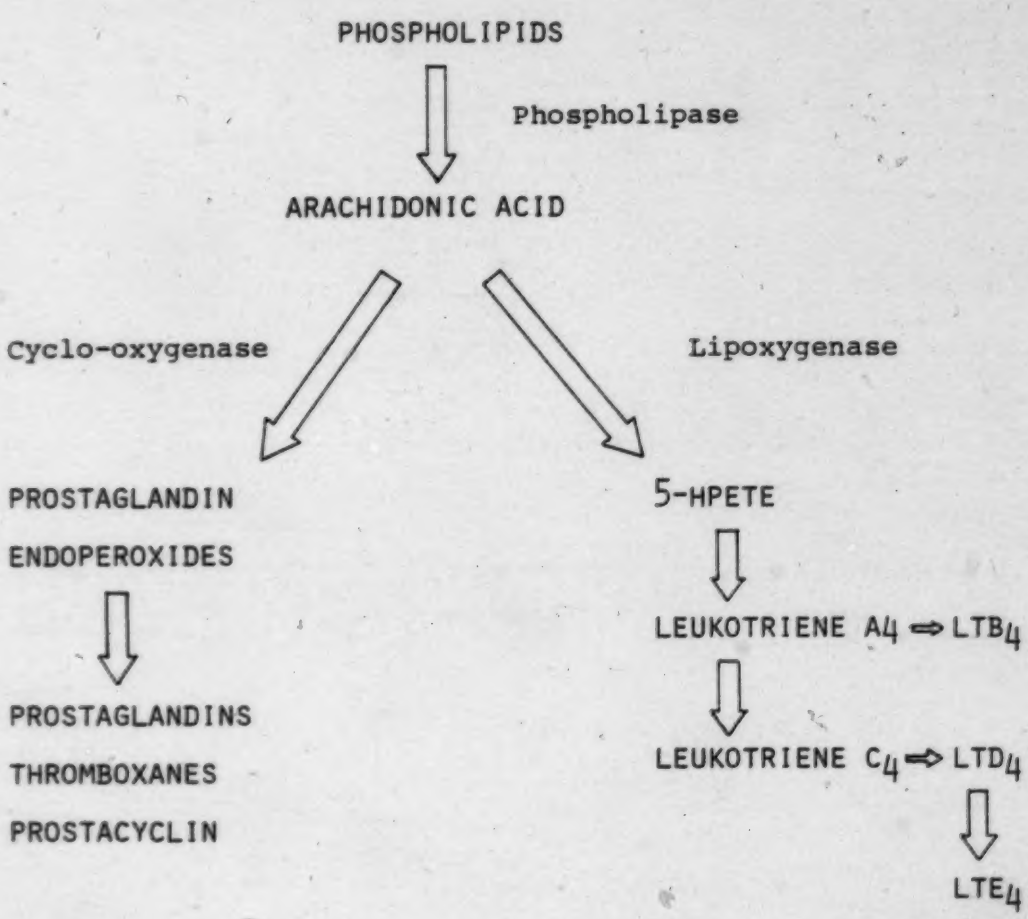
Prostaglandins(PGs) are synthesized, depending on availability of precursor chemicals, in most mammalian cells, where they are not stored but rather released immediately after synthesis. Several series of prostaglandins together with endoperoxides, thromboxanes, prostacyclin and leukotrienes are all derived from common substrates, and their actions are multiple. PGs released by the gallbladder modify motility,[33] fluid transport, mucus production and inflammation. Indomethacin; an aspirin-like drug, inhibits PG synthesis and is effective in relieving pain due to cholelithiasis.[34] Little is known about the influence of PGs on normal human gallbladder motility.

Biochemistry of Prostaglandins and Leukotrienes

These substances are all derived from phospholipids, the majority via arachidonic acid as depicted (Fig. 1). The arachidonate is directly ingested or derived from dietary linoleic acid, and becomes part of the cell membrane by esterification to phospholipids. Cleavage from the cell membrane by enzymes, mainly phospholipase A2, enables free arachidonate to be metabolized either via the cyclo-oxygenase or lipoxygenase system.

Since the rate of synthesis depends on phospholipase induced release of archidonic acid, the level of which is very low in the cell, this is the rate limiting step. The substrates produced are not stored in the cell but are released immediately and rapidly inactivated by

ARACHIDONATE PATHWAY



Cyclo-oxygenase + Lipoxygenase is Prostaglandin Synthetase.

FIGURE 1

widely distributed enzymes in the body tissues, especially in the lungs.

Whereas the cyclo-oxygenase system exists in most cell types, the lipoxygenases have been found in lung, platelets and leukocytes only, the resultant products being leukotrienes. These substances are mediators of hypersensitivity.

Inhibitors of the Arachidonate Pathway

Inhibition occurs at 4 sites

1. phospholipase A — example corticosteroids
2. cyclo-oxygenase — example aspirin and indomethacin
3. lipoxygenase — example nordihydroguaiaretic acid
4. final products — example imidazole

The therapeutic efficiency of anti-inflammatory drugs parallels their ability to block both the cyclo-oxygenase and lipoxygenase systems, and in this regard, corticosteroids are potent. Indomethacin and aspirin selectively block cyclo-oxygenase, indomethacin by reversibly interacting with the substrate binding site and aspirin by irreversibly inhibiting the enzyme by acetylating its amino terminal group.[35]

Physiological Actions

Prostaglandins are local modulators of biochemical activity, regulating blood vessel tone and response to hypoxia; platelet function; bronchoconstriction; uterine activity; renal tubular function; inflammatory response; oncogenesis and gastrointestinal function.

In the gallbladder the main effects are on mucus production, fluid transport and motility.

Mucin Production

The gallbladder epithelium is devoid of specialised goblet cells,

but it is well known that mucus production increases in gallstone disease.[36] These mucins come from the secretory granules of all the surface cells, especially those in the neck of the gallbladder and these glycoproteins may be responsible for nucleation of gallstones.[6]

Cholesterol induced gallstone disease in the prairie dog model is accompanied by increased secretory granule size and mucus hyperproduction and this is blocked by aspirin.[37] Furthermore, incubation of gallbladder epithelium with arachidonate stimulates mucin release and this can be inhibited by indomethacin, implicating prostaglandin regulation.

Fluid Transport

Bile concentration is an active process dependent on the sodium-potassium ATPase pump.[38] Prostaglandins modulate this function, mostly inhibiting fluid absorption.[39] However, in the diseased gallbladder, the normal absorption may be reversed to secretion, and this can be inhibited by indomethacin, again implicating prostaglandin involvement.[40]

Motility

The main hormonal mediator of gallbladder evacuation is cholecystokinin (CCK)[92], but it has been shown in human and animal gallbladders in vitro that prostaglandins can modulate tone and cholinergic induced contractile responses.[41]

Kotwall[33] found that the effect of exogenous prostaglandins on the human gallbladder in vitro depended on the stage of the disease process. Gallbladders with chronic mild and chronic advanced cholecystitis showed contractile responses to PGE_1 and PGE_2. Prostacyclin and leukotrienes had no effects.

Spontaneous contractile activity was again very variable, but the E group were again most likely to be implicated.[42] Prostaglandins may modulate tone and therefore flow into and out of the gallbladder.

Prostaglandin Release from the Gallbladder

The gallbladder releases prostaglandins in response to a number of stimuli. Damage to the mucosa, such as inflammation due to gallstones, releases arachidonate which potentiates prostaglandin synthesis.[43]

Indomethacin has been found to be effective in reducing biliary pain due to gallstones, prompting the authors to postulate that prostaglandins are responsible for fluid secretion, distension and potentiation of pain in humans.[44] Very limited information is available on the effect of prostaglandins on normal human gallbladder function.

PERSPECTIVES ON GALLBLADDER MOTILITY

The delivery of bile into the duodenum depends on a series of complex interactions between the hepatic secretion of bile and the motility of the cystic duct, gallbladder and sphincter of Oddi.

There are two major driving forces, the liver which secretes the bile at a pressure greater than the resting pressure in the biliary tree, and the gallbladder which intermittently contracts to expel its contents. A third motor component is the sphincter of Oddi with its putative phasic pump.[45]

The sphincter of Oddi provides resistance to biliary outflow and diverts bile into the gallbladder. In addition, there is strong evidence for sphincteric action in the cystic duct.[46,47,48]

Hormonal Control

Cholecystokinin (CCK), the most important mediator of gallbladder

emptying, is released from the I cells of the duodenum, jejunum and ileum in response to the products of digestion.[49] The active C terminal octapeptide is specifically immunogenic and invariably contracts the gallbladder muscle, probably via cyclic nucleotides directly. CCK may also be a neural mediator since this effect is partially antagonised by atropine in dog and opposum. Indomethacin blocks the CCK induced rise in cyclic GMP.[50]

Other effects of CCK include reciprocal relaxation of the sphincter of Oddi,[45] sensitive stimulation of pancreatic secretion[51] and choleresis.[49]

Gastrin and caerulein, hormones of the same family as CCK, cause gallbladder contraction in pharmacological doses. The secretin group of hormones may mediate relaxation. The only member of note in this respect is vasoactive intestinal polypeptide (VIP).[45]

Neural Control

The biliary tract is extrinsically innervated by the parasympathetic and sympathetic systems, via the vagus, coeliac and superior mesenteric ganglia.[52]

Cholinergic stimulation increases tone in the gallbladder and electrical stimulation of the vagus increases pressure in vitro. The rise in pressure is not associated with evacuation which occurs in a facilitated fashion only in the presence of CCK.[50]

Sympathetic control via stimulatory α adrenergic receptors or the more important inhibitory or relaxant β receptors is controversial. The adrenergic influence may be more pronounced on the sphincter of Oddi and cystic duct.[53]

The presence of non adrenergic, non cholinergic inhibitory nerves

in the biliary tree was suspected following reports of atropine resistant vagally induced relaxant responses in the bowel.[54] These effects have been shown in the gallbladder and sphincters, and are proposed to be due to a purine neurotransmitter. These nerves may mediate receptive relaxation.

The Effect of Vagotomy on Gallbladder Function

Several studies have suggested that total vagotomy leads to dilatation of the gallbladder[55,56] and formation of stones.[57,58,59,60] More refined surgical techniques which spare the pyloroduodenal mechanism and hepatic innervation are resistant to these changes.[61]

Animal studies show conflicting results. Passage pressures across the cystic duct and sphincter of Oddi decrease intraoperatively in response to vagotomy in the dog,[62] but gallbladder pressure increases postoperatively with no change in rate of emptying.[63] In the rabbit, gallbladder pressure decreases,[56] and in both species, dilatation results.

Human cholesterol gallstones implanted into dogs dissolve more slowly[64] and produce cholecystitis following vagotomy.[62]

In humans, Shaffer showed a decrease in lithogenicity following vagotomy, but no change in function, or partitioning of hepatic bile. However, nuclear scintigraphic techniques do not quantitate volume, and so a change in gallbladder compliance could account for these findings.[65]

The Pyloro Cholecystic Reflex

In the dog, antral distension has been shown to produce gallbladder evacuation.[130] The mechanism is blocked by atropine and vagotomy and is independent of serum gastrin. However, the temporal relationships of

this reflex are not stated and it is not known if this reflex occurs in man.

PERSPECTIVES ON ULTRASONOGRAPHY

Ultrasound is the term used to describe sound when the pitch is too high to be to be audible.[66] It is now a well accepted, clinically valuable diagnostic modality, but there was a time when it was poorly understood and the results were viewed with skepticism.

History[67]

Natural use of echolocation by bats was observed by Spallanzani in the 18th century - but humans lacking a biological source of ultrasound awaited the discovery of the piezolectric crystal by Pierre Curie to enter the field.

Piezoelectric crystals which produce mechanical vibrations when deformed by an electric field, or vice versa, serve as transducers. The frequency of the vibration depends on the physical characteristics of the crystal.

A French engineer, Paul Langeuvin, observing that sound travelled better in water than air, is credited with the work leading to Sonar (Sound Navigation and Ranging). This was expanded following the war to therapeutic diathermy and cancer therapy with high energy ultrasound.

The prototype scanners built in the late 1950's led to Dr. Ian Donald imaging the foetus in a "sea of amniotic fluid", and the development of echocardiography by Edler and Hertz in Sweden.

Kossoff introduced gray scale imaging some 15 years ago, with further technological advances coming in rapid succession.

Physics[67]

Sound is the transmission of mechanical vibrations in a pulsed oscillatory motion through a medium. Audible sound is in the range 20 Hz to 18 KHz, while ultrasound has a greater frequency. The possible frequencies range from 1 Hz to 10,000 MHz, but the practical range is 1-15 MHz. Ultrasonic echoes are derived from acoustic interfaces, due to reflection and refraction. The degree of reflection depends on the difference in acoustic impedance of the tissues, given by

$z = p \times c$ where z = acoustic impedence

p = density of the tissue

c = speed of sound in the tissue

Since the density and therefore the speed of the wave through bone, gas and soft tissues are very different, large z values are produced. Thus a soft tissue to bone or gas interface reflects most of the impulse, producing an "acoustic mirror" and blocking transmission to deeper structures, irrespective of the amplitude or power of the impulse.

Resolution is the ability to separate two interfaces, and depends on the frequency (axial resolution) and the beam width (lateral resolution). The axial resolution is the resolution along the path of the sound beam and theoretically for transducers of 3.5 MHz, this is 0.44 mm, and for 5 MHz it is 0.31 mm.[66] Unfortunately the equipment does not reach these limits, and in addition as frequency and therefore resolution increases, so does the attenuation of the sound.

Lateral resolution refers to resolution perpendicular to the axis of the sound beam and practically is in the region 2 to 3 mm.[66]

Safety of Ultrasound

High intensity ultrasound waves can permanently alter the tissues through which they pass, the effects varying from warming to total destruction. The power levels required to produce these effects are many orders of magnitude greater than those used in diagnostic scanning and no commercially available equipment reaches the arbitrary limit of 100 mw/cm^2.[68]

Clinically, no serious biological effects have been demonstrated, and in the world literature there are no substantiated reports of biological effects from ultrasound at diagnostic levels. Experimentally, ultrasound is capable of causing sister chromatid exchanges, considered an indicator of gene mutation and carcinogenesis.[69]

The recommendations of various groups concur that

1. Ultrasound is of great benefit to modern medicine.
2. Ultrasound apparently has no immediate deleterious effects.
3. Further investigations regarding the safety of ultrasound are necessary.
4. Ultrasound should be used judiciously only when necessary (refering mainly to the foetus).[69]

The uncertainties relate to long-term effects, and the additive effects of ultrasound and ionising radiation.[68]

Very little ultrasound occurs naturally, and thus the effects are difficult to assess in the long term.

Real Time Ultrasonography

Although real time ultrasound has been available since 1960, lack of resolution has limited its role. However, the increasing

sophistication of the equipment led to general use by 1978, and by 1980 it was clearly superior to the static scanner in many areas.

The advantages of real time are speed, flexibility, resolution, diagnostic utility and it is probably the easiest form of sonography for the novice to use.

The term 'real time' arises from the computer world and refers to the ability of the computation to keep up with the process it is measuring. Neither the static nor the real time scanner provides absolute positional information with respect to the body, except that which is derived from a knowledge of anatomy. However, some machines are capable of indicating the position of a scan section relative to a section previously coded.

The sensitivity of an instrument as measured by the ability to detect small, distant or weakly reflecting objects is dependent on the acoustic intensity or the acoustic energy that must be transmitted. Until recently, manufacturers have been reticient to make the intensity data known, but for linear array equipment, the spatial-peak temporal average (SPTA) intensity is less than 0,12 mW/cm^2 as opposed to B scan of approximately 45 mW/Cm^2. Thus it is obvious that the linear array projects extremely low intensities.

Ultrasonography of the Gallbladder

Since 1924, the oral cholecystogram has been the diagnostic standard by which investigations of the gallbladder have been judged. However, with the advent and improvement of ultrasound, this technique has been prefered in emergency and elective cases, and its sensitivity and specificity are at least as high if not higher than oral cholecystography.[70,71]

Anatomy

The gallbladder is a fluid filled, pear shaped structure, variable is its position in the body, although situated predominantly in the right upper quadrant. The fundus can occasionally extend into the right iliac fossa or left side of the abdomen, but the relationship of the body of the gallbladder to the liver is fixed. The gallbladder bed is situated along the junction of the medial segment of the left lobe (quadrate lobe) and the right lobe of the liver. The neck of the gallbladder points towards the porta hepatis and is connected by the main lobar fissure to the region of the portal vein.[72] The gallbladder is frequently folded upon itself. The fold may not be fixed and may change with rotation of the patient. In other cases adventitial adhesions can cause fixation of the fold, which appear as septa within the gallbladder. With a fold at the fundus, the distal segment is known as a "phrygian cap",[73] and if at the neck, the proximal portion is called "Hartman's pouch".

Ultrasound Technique[74]

The main advantage of real time over static scanning is the ease and rapidity with which the gallbladder can be found. Longitudinal and transverse scans are made, and a "slow walk" will demonstrate the whole interior, and rule out the presence of small calculi. Patient position can be varied from supine, to left posterior oblique, sitting or standing to give optimal views.

Cholelithiasis

The sonographic appearance of a calculus is a strong echo from the bile-calculus interface with an acoustic shadow.[75] It used to be thought that the presence of a shadow was dependent on the makeup of the

stone, but it has now been shown that the shadow appears when the majority of the sound beam is intersected by the calculus. Thus it depends on the size of the calculus and the focusing of the beam.[76]

Calculi are sought especially in dependent areas - mobility (the rolling stone sign) may differentiate these from abnormalities of the gallbladder wall.[74]

Acute cholecystitis

Although calculi are diagnosed accurately, their relationship to the symptoms can be clinically difficult to determine. Gallbladder wall thickening[77] and a gallstone fixed in the neck are hard signs. In addition, pressure with the transducer on the gallbladder area producing pain - the "specific Murphy's sign" is helpful.

Echogenic Bile

Normal bile is echo free, but if stasis occurs, as in prolonged fasting, the bile can become diffusely echogenic. This is due to the presence of crystals in the bile.[78]

Floating Stones

Cholesterol gallstones are denser than bile, and only float if the specific gravity of the bile is increased, e.g., with contrast materials.[79]

Measurement with a Real Time Scanner

Measurements depend on the assumption that the sound travels at uniform speed, which it does not although the differences are small.

Dimensions of structures can be measured from ultrasound images with the use of accurate electronic calipers. The overall accuracy of any measurement is much more dependent on subjective sources of error,

for example, uncertainty in the selection of the dimension to be measured. Overall the error in a careful measurement of length is approximately 2%.[66]

From ultrasonic images, cross-sectioned areas can be measured with an error of around 5%. From a number of cross-sectional areas, a volume may be derived.[66]

Ultrasound has been used to measure the volume of numerous organs, such as the gallbladder,[80] liver,[81,82] spleen,[83] urinary bladder, prostate,[84,85] foetal skull,[86] foetal growth sac,[87,88] muscle compartments and ventricular volume in addition to measuring the volume of pathological structures such as gallstones, tumour masses, pleural effusions, pericardial effusions, subphrenic and other abscesses.

Estimation of Gallbladder Volume

The gallbladder varies in size and shape, lying under the liver in contiguity with numerous other abdominal viscera. The volume is extremely awkward to measure in vivo.

The shape of the gallbladder is dependent on the physical characteristics of the wall, and the varying tensions developed in it, together with the external forces on the walls from the abdominal contents. In addition, a variable portion is in continuity with the liver, and thus relatively fixed.

The original methods for estimating volume were projections from the two dimensional oral cholecystogram image.[89] The estimations made have been used in numerous articles although never completely validated in the human.[90] These methods were expanded for used with the echotomogram[80] which has the advantage of mobility enabling rapid imaging in several planes.

The volume of the gallbladder may be estimated by

1. Cylindrical Approximation

   The gallbladder is considered to be cylinder with length and cross-sectional area

   $V = l.\pi r^2$ or $V = \frac{\pi d^2 l}{4}$

2. Ellipsoid Approximation[91,92,93]

   The cross-sectional area is considered to have 2 dimensions, height and width.

   $V = \pi \times \frac{h}{2} \times \frac{w}{2} \times l$

3. Corrected Ellipsoid Approximation[94]

   A correction factor is introduced to allow for the pear shape of the gallbladder.

   $V = 1.1 + 0.6427 \left(\pi \frac{h}{2} \times \frac{w}{2} \times l\right)$

   The correction factor was calculated from the regression of the Ellipsoid Approximation on the volume calculated by the Sum of Cylinders method.

4. Sum of Cylinders[89]

   The gallbladder is considered to be made up of numerous cylinders of equal height and the volume of these is summed

   $V = \sum \frac{\pi\, di^2\, hi}{4}$

5. Corrected Sum of Cylinders[80]

   A correction is added to account for non-linearity of the gallbladder (vide infra).

   $V = 0.785 hE^2 \left(\sum di^2\right)$

6. Simpsons Formula for Numeric Integration[95]

This method was derived for use with radiographs in 2 dimensions, and is adapted for use with ultrasonography. The gallbladder is considered to have length (ℓ) and cross-sectional area (a_1) at points 1/6th of ℓ.

$$V = \frac{\ell}{18}(4a_1 + 2a_2 + 4a_3 + 2a_4 + 4a_5)$$

Most of these methods derive from the two dimensional views obtained with oral or intravenous cholecystograms. The methods were either simple mathematical derivations or based in part on the sum of cylinders method of Siffert de Paula[89] as popularised by Boyden.[96] Methods based on ultrasonic technology are limited.[97] Everson and colleagues derived a unique method based on a shrewd concept.[80] Real time (linear array) ultrasound produces an echotomogram in two dimensions. The geometric longitudinal axis of the gallbladder, however, is almost invariably curved, often in a compound fashion (Fig. 2). Thus, the longitudinal ultrasonographic plane will not pass along the geometric longitudinal axis, causing the antero-posterior (A.P.) diameter measured to be smaller than the true diameter.

To correct for this a correction factor, E, was introduced[80] Measurement of this factor requires imaging the gallbladder in transverse section to determine the true AP diameter and width.

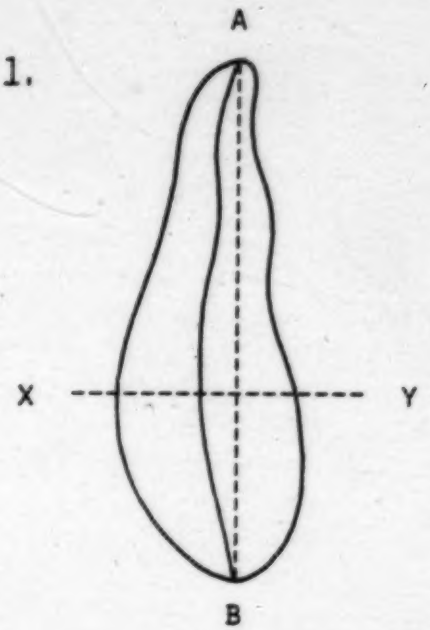
$$E = \frac{\text{A.P. diameter + width}}{2} \times \frac{1}{\text{greatest diameter on longitudinal projection}}$$

$$\text{or } E = \frac{h + w}{2} \times \frac{1}{d}$$

Studies using this method - the corrected sum of cylinder method, to estimate gallbladder volume compare well with estimations from oral

PROJECTIONS OF THE GALLBLADDER.

1.



RADIOGRAPHIC SILHOUETTE AS MIGHT BE PRODUCED BY THE P.A. PROJECTION OF THE GALLBLADDER. THE GEOMETRIC LONGITUDINAL AXIS IS INDICATED BY THE SOLID LINE A-B, AND THE ULTRASONOGRAPHIC LONGITUDINAL PLANE BY THE DOTTED LINE A-B.

2. THE TRANSVERSE CROSS-SECTION X-Y IS SHOWN. THE GEOMETRIC LONGITUDINAL AXIS BY DEFINITION OCCUPIES THE MID-POINT OF THE MAXIMUM P-A DIAMETER. HOWEVER THE ULTRASONOGRAPHIC LONGITUDINAL PLANE FALLS LATERALLY ON THE SHORTER LINE C-D.

FIGURE 2.

cholecystograms and with in vitro models.[80] The method has not been validated in vivo, in animals or humans.

Gallbladder Function Studies

Gallbladder function can be studied by physiological or morphological means, or by a combination of both. The physiological mechanisms are mucosal absorption of water and electrolytes which concentrates stored hepatic bile and smooth muscle contraction which discharges it.

The normal constituents of bile provide a means of studying function under certain conditions,[98] as do absorbed substances such as Indocyanine Green, secreted by the liver and measured in the biliary effluent. These methods are accurate and have provided important information,[99] but do require duodenal intubation.[17]

Gallbladder function is required to produce an activity image for nuclear scintigraphy,[100,101] and also to produce a morphological image for oral or intravenous cholecystography. Historically, oral cholecystography is the gold standard by which other modalities are judged,[102] and much of the work on normal and pathological function in humans has involved radiology. Ultrasound does not depend on function and is purely morphological.

The ideal function study should be physiological, safe and painless, the method should be standardised and sensitive and the results accurate and reproducible.

Preparation

A period of fasting is normally required to ensure that the gallbladder fills. The gallbladder may begin to fill as early as 30 min following a test meal,[103] but a period of 8 hrs is normally allowed, the

gallbladder filling scintigraphically at 2% per minute.[104]

Meals of different composition and therefore variable gastric emptying may retard filling, and in addition the influence of olfactory and cephalic stimuli is uncertain.[96]

During or preceeding the fast, labels of radio-opaque, chemical dyes or radioactive tracers are given attached to suitable carriers, the possible influence of these on normal motility is unknown.[105]

The migrating myoelectric complex (MMC) is propagated through the bowel at regular intervals in dogs,[106,107] and there is good evidence to show an associated biliary secretory phase in humans.[108,109] Gallbladder evacuation of up to 30% is documented,[108] in association with the MMC, which can begin within 5 hrs of the last meal in certain cases.

Stimulus for Contraction

CCK produces dose dependent contractions of the human gallbladder,[110,111,112] and can be administered parentrally or its release stimulated with intraluminal agents.[94,92]

Intravenous CCK has the advantage of accurate quantitation and immediate effect,[113] but still lacks standardisation of source, purity, chemical composition and method of administration.[114] In addition, the effects can vary biologically due to end organ sensitivity.[115,116]

CCK release can be stimulated by numerous agents and these can be administered orally[80,117] or by tube into the stomach or duodenum.[17] The modifying influence of such a tube has not been clarified.[104]

Boyden, in keeping with his time, used egg yolk and cream in extensive studies,[96] but derived foodstuffs and pharmacological agents[118] have dominated the recent literature.

Gastric emptying is influenced by numerous factors such as solid matter,[119] fats, osmolality[120] and volume of the meal.[121,122] None of these factors is standard in the literature.

Measurement

In order to assess gallbladder emptying the volume of the gallbladder need not be known.[112,123] Changes from an arbitrary zero point can be measured with dilution techniques[17] or reduction from maximum activity in the various types of nuclear scan.[113,110,23]

From cholecystographic and ultrasonic images, changes can be measured in the same way, the initial volume not necessarily being known. In this case, the change in cross-sectional area or volume by any of the methods described can be represented.

Interpretation

To quantitate gallbladder evacuation, 3 basic parameters have been used.

1. An allocated zero point measure, either as 100% or as a calculated volume or concentration.
2. A variety of rate constants, based on the best fit to a certain function or integrals or a slope.
3. The percentage change in size or activity during a fixed time period from the stimulus.

To obtain the rate constants, the data have been analysed by the best fit to linear, logarithmic,[124] power[65] and exponential curves[129]. The type of function is arbitrary, but the limits are critical, and the criteria by which these analyses are performed is often inadequately described.

Given the methodology, it is difficult to compare the results in

the literature. Gallbladder function is known to be variable,[126,127] and the range of normal remains to be resolved.[114]

Normal Gallbladder Function

Following an overnight fast, resting gallbladder volume remains approximately constant except when associated with the MMC. Stimulation with intravenous CCK, (following a lag period) produces rapid evacuation. The lag period has not received consideration in the literature.[104] Studies by Boyden with radiography, showed an abrupt decrease in gallbladder volume following a test meal. This stopped approximately 2 min later, to continue again shortly thereafter. This feature was dubbed the "2 minute pause" and was thought to be due to asynchronous closure of the spincter of Oddi.

Evacuation of the gallbladder proceeds in a log linear fashion[128] for a period and then slows or stops. Most authors agree that the majority of the contents have been expelled in 30 mins[126].

A mixed solid diet results in continued stimulation and minimal refilling of the gallbladder which remains in a contracted state until the overnight fast.[129]

Considering the multitude of variables which control the phases of gallbladder filling and evacuation, it is not possible to fully evaluate the gallbladder function parameters.

The Future in Ultrasound[66]

Little progress is anticipated with A & B mode static scanners, but developments in digital scan converters and image processing, with a large enough computer to store and reprocess the raw data from multi-directional scans would enable real time computerised tomography (CT).

Ultrasonic CT has the theoretical advantage that not only the

attenuation of the beam can be processed but also the reflections. This has been used for small parts such as limbs and breasts which can be immersed in water.

With progess in single crystal transducers, parallel advances are being made with multi-element transducers, particularly by improving resolution with electronic focusing.

Developments in holography and tissue characterisation are slow, and modalities such as nuclear magnetic resonance and positron emission tomography remain to be evaluated.

OBJECTIVES

1. To review the methods and compare the accuracy with which the volume of a gallbladder may be estimated in situ.
2. To use the most suitable method to estimate the changing volume and determine its limitations in vivo.
3. To develop and standardise a human gallbladder function study.
4. To derive and define functional parameters so that quantitative comparisons can be made and to determine the normal variations.
5. Using the function study, to determine the effect of indomethacin administration on normal gallbladder function.

The Present Study

This was conducted in 4 parts

1. Ultrasonographic estimation of gallbladder volume - a human post mortem study.
2. Ultrasonographic estimation of changing gallbladder volume - an animal study.
3. Evaluation of normal human gallbladder function; determination of functional parameters and variations.
4. Investigation of the influence of indomethacin on normal gallbladder function.

MATERIALS AND METHODS

The portable ADR 2130 grey scale real time scanner (Advanced Diagnostic research, Tempe, Arizona USA) was used in all parts of this study.

Two hand held linear array transducers were utilised, the 8 cm, 5MHz for the animal studies and the 12 cm, 3.5 MHz for human imaging.

The scanner's oscilloscope with storage facility was fitted with a Polaroid (Polaroid Corporation Cambridge MA02139 USA) camera focused directly onto the screen and operated by a remote pedal. Hard copy was recorded on Polaroid 667 Coaterless Land pack film (Polaroid (UK) Ltd. St. Albans, Hertfordshire, England).

Proficiency in the use of real time ultrasound on the gallbladder was obtained with the help and guidance of the Radiologists and Ultrasonographers of the University of Alberta Hospital. Loan of the equipment used was kindly authorized by Dr. S. Wilson.

The hard copy was analysed initally with the Apple II C personal computer (Apple Inc. California) on the Apple graphics tablet with matched commercial digitizing software. Subsequently, a Microplan II microprocessor (Laboratory Computer System Inc. 139 Main St., Cambridge, MA 02142) with printer (Microline 80 Matrix Printer OKl electric industry company Ltd., Mt. Laurel, New Jersey) was used which directly determined area, maximum diameter, maximal length and form factor together with a statistical analysis of these data. (Appendix 1)

All calculations, regressions, basic statistics and statistical analyses were performed on a Hewlett Packard (H.P. 97) calculator.

1. POST MORTEM STUDY

Cadavers were studied at the University of Alberta Hospital Autopsy suite with approval of the Department of Pathology and the Pathologist of the day, and the cooperation of the Autopsy Technicians.

Prior to routine autopsy each candidate was screened and excluded if there was evidence of cholecystectomy, recent laparotomy or local conditions which would prevent imaging. A total of 25 cases were obtained but 17 were rejected leaving 8 which were suitable for evaluation.

Cases were excluded for:

Non-visualisation of the gallbladder - 13 cases

3 previous cholecystectomy

2 fibrotic shrunken gallbladder with stones

3 scars or recent laparotomy obscuring view

2 massive ascites

1 gross obesity

2 carcinomatous metastases in liver

Technical Errors - 4 cases

1 inadequate ultrasonographic technique

2 bile spillage on removal of gallbladder

1 camera failure

Imaging of the Gallbladder

The right upper quadrant was repeatedly scanned, acoustic coupling being maintained with ultraphonic S.G. oil (Pharmaceutical Innovations inc. Newark, New Jersey).

Scans were made initially in the sagital plane to image the ultrasonographic longitudinal plane of the gallbladder as adjudged by the electronic calipers. Images on the storage oscilloscope were measured and recorded on film.

The transducer was then rotated 90°C and multiple images in the maximum transverse plane similarly recorded.

Removal of the Gallbladder

The viscera were removed in the prescribed manner, en block via a midline incision from hyoid to pubis. Very little pressure was applied directly to the viscera. The investigator clamped the cystic duct at the neck before the pathologist separated the thoracic from the abdominal viscera. The gallbladder was removed by sharp dissection and excess connective tissue debrided. The case was excluded if any spillage of bile occured.

Measurement of Gallbladder Volume

The whole specimen was measured by fluid emersion in a specially modified calibrated cylinder. The inital fluid volume, volume with whole submerged gallbladder and residual volume after removal of the organ were recorded on the proforma. The gallbladder contents were then expressed and measured directly.

Calculation of Gallbladder Volume

The volume in each case was calculated manually with a calibrated grid and measuring rule by 6 methods, representing the types of estimations from the literature (see introduction p. 19.)

Analysis of Data

The calculated volume, in each case, by each method, was compared

to the actual measured volume of the gallbladder, the coefficients of correlation calculated by linear regression analysis, and the corresponding p value derived.

In each case, the percentage error of the calculated volume from the actual volume was found, and the arithmetic mean of these errors expressed as the mean percentage error of the method.

2. ANIMAL STUDY

The adult mongrel dog served as a chronic model for in vivo estimation of gallbladder volume and changes in volume.

Animals in the study were used in accordance with the principles contained in the Care of Experimental Animals, Canadian Council on Animal Care.

Operative Technique

Under general anaesthesia with Somnotol (pentobarbital) 30 mg/kg i.v. and oro-tracheal intubation, a midline abdominal incision was made, the cystic duct isolated, ligated proximally and cannulatd with a modified 6.F. Fogarty catheter (Edwards Lab. Inc., Santa Ana,California). The cannula was secured with a 2/0 silk ligature and the distal end exteriorised into a subcutaneous pocket with the guide wire obturator locked in situ.

The abdomen and subcutaneous pocket were closed with 2/0 Dexon (polyglycolic acid, Davis & Geck, Cynamid, Montreal) in layers. A recovery period of 10 days was allowed before the first test.

Test Procedure

Anaesthesia and intubation as previously ensured optional conditions for the test. The belly was closely shaved to allow acoustic

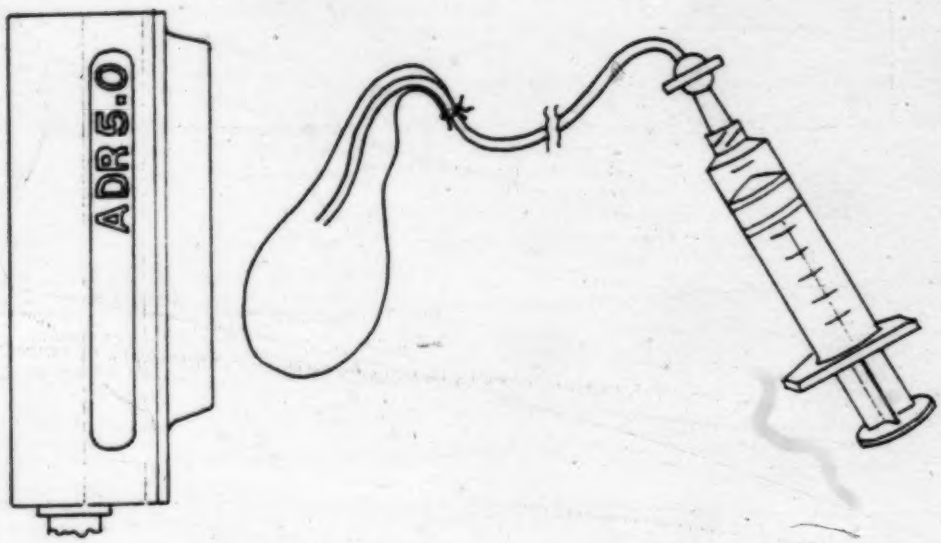


DIAGRAM OF ANIMAL MODEL:

THE VOLUME OF THE GALLBLADDER
WAS CHANGED BY KNOWN INCREMENTS
AND SIMULTANEOUSLY IMAGED.

FIGURE 3.

coupling with ultraphonic S.G. oil to the 5MHz transducer. The gallbladder was imaged in the longitudinal plane only as the size of the dog and the narrow subcostal angle precluded an adequate transverse image.

The fogarty luer lock was mobilised from the subcutaneous pocket and via a three way stopcock, emptied of accumulated debris and syringed until clear. The system was primed with 25 ml of normal saline (Fig. 3).

Data Collection

The assistant introduced known aliquots of saline from the syringe, the investigator blinded to the volume used, imaged and recorded on film the longitudinal cross section of the gallbladder.

The hard copy was measured manually and the gallbladder volume estimated by the sum of cylinders method. The longitudinal area was measured by digitization on the Apple IIc graphics tablet.

Analysis of Data

The calculated volumes were compared to the known volumes, analysed by linear regression, and the appropriate p values derived.

3. NORMAL HUMAN GALLBLADDER FUNCTION STUDY

The protocol for parts 3 and 4 of this study were approved by the Ethics Committee, University of Alberta Hospitals October 25, 1983.

Normal male volunteers (n=18) aged betwen 22 and 61 yrs (mean 31 years) were interrogated for history or symptoms of liver or biliary disease, and informed consent was obtained.

The stimulus for gallbladder evacuation was a standard liquid meal consisting of 2 whole medium eggs, 200 ml full cream milk, 2.5 g refined sugar and 100 g banana, homogenised for 5 minutes in a blender at 1500

R.P.M.

Each individual presented on at least one occcasion, after a 12 h overnight fast. One volunteer presented on 8 occasions.

Collection of Data

The gallbladder was located in the right hypochondrium and a record was made in each case of the maximum longitudinal cross-section. Transverse scans were not made.

The test meal was ingested at the time allocated zero. Images were recorded at approximately 2 min intervals to 20 min, 4 min to 40 min and 5 min intervals for 70 to 90 min.

Any reaction to the meal such as a gastrocolic reflex, nausea, flatulence or borborygmi were noted, as was passage of gastric contents through the duodenum seen on the scan

Analysis of Data

The areas were measured by digitization on the microplan II microprocessor with printout of data.

The data was fitted to one of four possible functions; linear, exponential, logarithmic and power curves; and the closeness of fit determined by the least squares method.

type of fit	'r' to 30 min	'r' to 80 min
linear regression	0.95	0.87
exponential curve fit	0.96	0.91
log curve fit	0.97	0.93
power fit	0.97	0.95

By convention, the changing area was plotted as a function of time by the best fit to an exponential curve (Fig. 4,5,6).

From the data and the curve described, the following parameters were recorded

A rest: the mean area of the gallbladder for the 10 min prior to stimulation.

A max: the maximum recorded area of the gallbladder following stimulation.

b_o: the overall rate constant of emptying.

b_1: the rate constant of emptying of the initial or rapid phase.

b_2: the rate constant of emptying of the second or slow phase.

tb_1 the time period of the initial phase of emptying.

%E30: the percentage change in area in 30 min following stimulation.

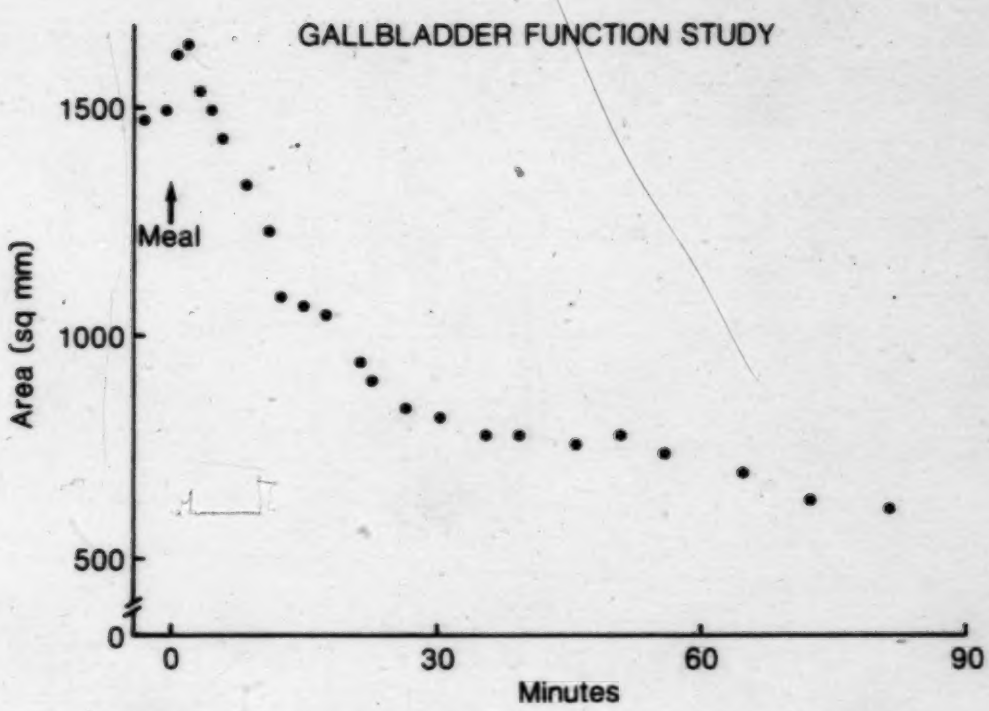
t lag: the lag period between stimulation and the onset of evacuation of the gallbladder.

For details of analysis - see Appendix 2

4. THE INDOMETHACIN STUDY

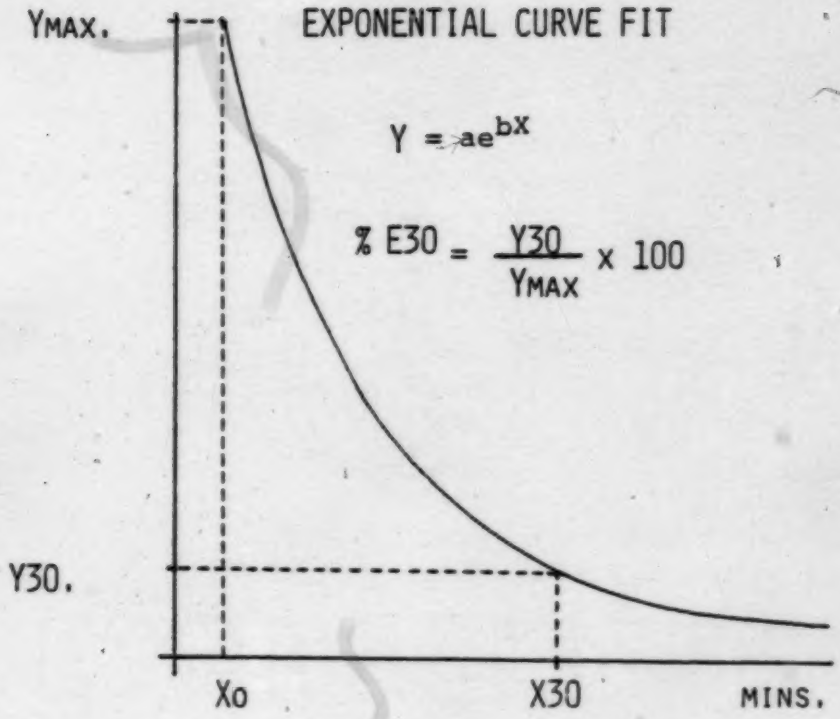
The possible effects of inhibition of prostaglandin synthesis on normal gallbladder function was studied in 2 parts.

The volunteers obtained were interrogated for symptoms related to the gastrointestinal tract, particularly peptic ulcer symptoms or possible allergic reactions to anti-inflammatory drugs, and informed



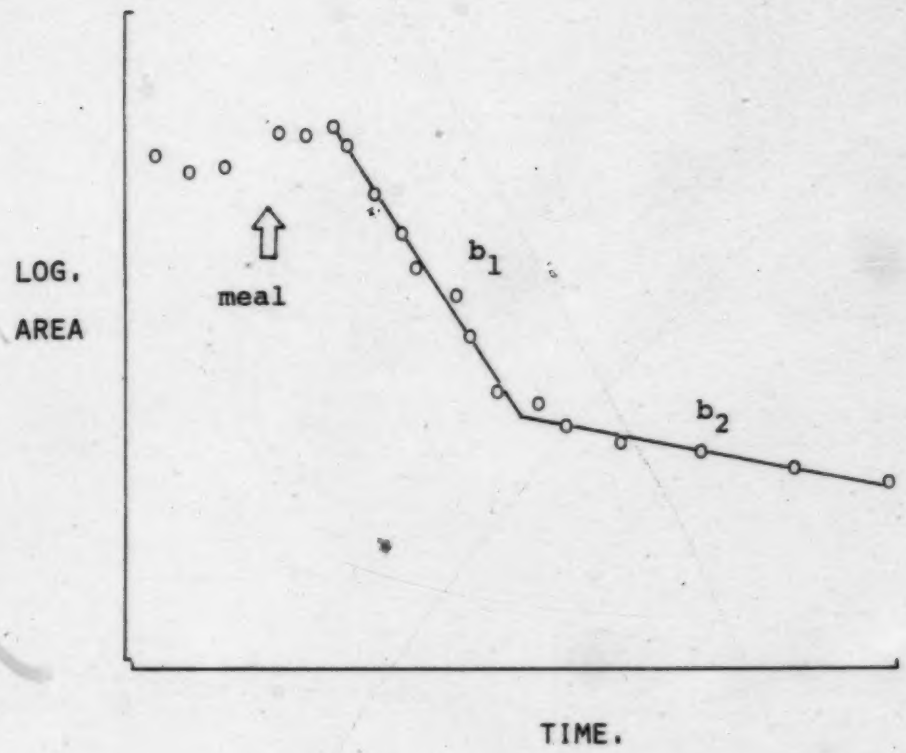
THE LONGITUDINAL CROSS-SECTIONAL AREA OF THE GALLBLADDER MEASURED BY DIGITIZATION FROM THE ULTRASONOGRAPHIC IMAGE, PLOTTED AGAINST TIME.

FIGURE 4.



THE AREAS MEASURED FROM THE IMAGES OF THE GALLBLADDER ARE FITTED TO AN EXPONENTIAL FUNCTION BY LEAST SQUARES.

FIGURE 5.



The log. of the areas plotted against time shows the two log. linear phases of emptying, with separate rate constants, b_1 and b_2.

FIGURE 6.

consent was obtained.

Collection of Data

In the first stage, 8 healthy male volunteers were studied before and after a 4 day course of indomethacin (Indocid Merck-Frost) 100 mg per day in 4 divided doses. Volunteers were instructed to take the medications with meals to lessen possible gastric irritation.

Five of these volunteers were restudied on no medication at least 3 weeks later. The volunteers served as their own controls.

In the second stage, 6 healthy male volunteers (4 from the first stage) were studied following either an indomethacin suppository, 100 mg (Indocid-Merck Frost) or a placebo of 1.5 gm (Pharmacy - University of Alberta Hospitals). of the carrier only ("Carobowax" suppository base A, polyethylene glycol 400), as to which the volunteer was blinded. Following self administration of the suppository, the resting gallbladder was imaged at 5 min intervals for 30 min, before a function test was performed in the standard manner. Any symptoms, abdominal cramps, nausea or tenesmus were noted.

Analysis of Data

The functional parameters derived as described were compared using the paired Students t test, p values < 0.05 being considered significant.

RESULTS

1. Human Post Mortem Study

In each case, the volume calculated by one of the 6 methods described was compared to the measured volume of the gallbladder by linear regression analysis. All methods showed good correlation, but poor accuracy (Figs. 7-13) (Table 1).

The estimation of volume by the corrected sum of cylinders method produced the lowest mean error (19%) and the highest correlation, ($r=0.91$).

The various methods tended to either over or underestimate the actual volume, and in this regard, the cylindrical approximation method and the ellipsoid approximation corrected to the sum of cylinders method showed the closest correspondence of line of identity to regression line.

Overall, no method was clearly superior, and the longitudinal cross-sectional area correlated nearly as well ($r=0.75$) as most of the more elaborate calculations.

The methods of calculation of gallbladder volume were divided into two groups, depending on whether one or two images were used in the calculation.

		r	mean % error
2 Dimensions:	Longitudinal Area	0.75	-
(single image)	Cylindrical approx.	0.71	32
	Sum of cylinders	0.73	38
	Simpson's formula	0.73	39
3 Dimensions:	Ellipsoid approx.	0.87	66
(two images)	Corrected ellipsoid approximation	0.86	24
	Corrected sum of cylinders	0.91	19

2. Animal Study

The dog tolerated the procedures well although the gallbladder was noted to be tense and full of tenacious mucinous debris prior to syringing and priming.

The system was primed with 25 ml of normal saline and at the conclusion of the experiment, 26.5 ml was withdrawn indicating that some secretion had probably taken place but that leakage was unlikely.

The catheter blocked and dislodged on one occasion but at replacement and subsequent autopsy there was no evidence of leakage or peritonitis. The gallbladder was moderately inflammed following the first operation, but had settled by the time of the test.

The longitudinal cross-sectional area and the volume calculated by the sum of cylinders method was compared by linear regression analysis to the known volume (Figs. 14,15, Tables 2,3). The mean error of the area and the volume from the regression line was 7%. However, the overall mean error of the calculated volume from the true volume was 59%.

3. Normal Motility Study Results

The data obtained in each case was analysed as described and the function parameters obtained (Fig. 4,5,6, Appendix 2).

The mean, standard error of the mean and coefficient of variation percent (CV%) were calculated for each parameter (Table 4).

A large variation was found between the 18 normal individuals.

The individual tested on 8 occasions again showed considerable variation in the parameters (Table 4).

The most consistent parameters between and within individuals was Amax and %E30.

Linear regression analyses were performed between the parameters to test for interdependence.

A significant negative correlation was found ($p < 0.05$) between b_1 and tb_1 (Table 5) and a positive correlation between b_1 and %E30, and b_o and %E30, but all other regressions failed to reach significance.

It was noted, however, that A max was consistently larger than A rest. This was significant ($p = 0.0004$) on a paired t-test analysis. In addition, the form factor showed an increase over the period of stimulation ($p = 0.02$), indicating that the images tended to become rounder at that time.

4. Indomethacin Study Results

In the oral indomethacin (21 tests) and the rectal indomethacin (12 tests) studies, the function parameters were calculated as previously (Appendix 2) in each case.

1. Oral Indomethacin

All volunteers completed the course of the drug in full and only 2 reported possible side effects (mild visual disturbance and

lethargy). There was no noted change in lifestyle due to drug administration, dysepsia, rashes or anorexia. The period of fasting and timing of the test was the same in each individual pair.

To test for homogeneity, a one way analysis of variance was performed comparing the controls with the test populations for A max, b_o and b_1. Since no difference was found, the mean of the two controls was compared with the test.

The parameters measured (Table 8) were compared control vs test drug by a paired Students t test.

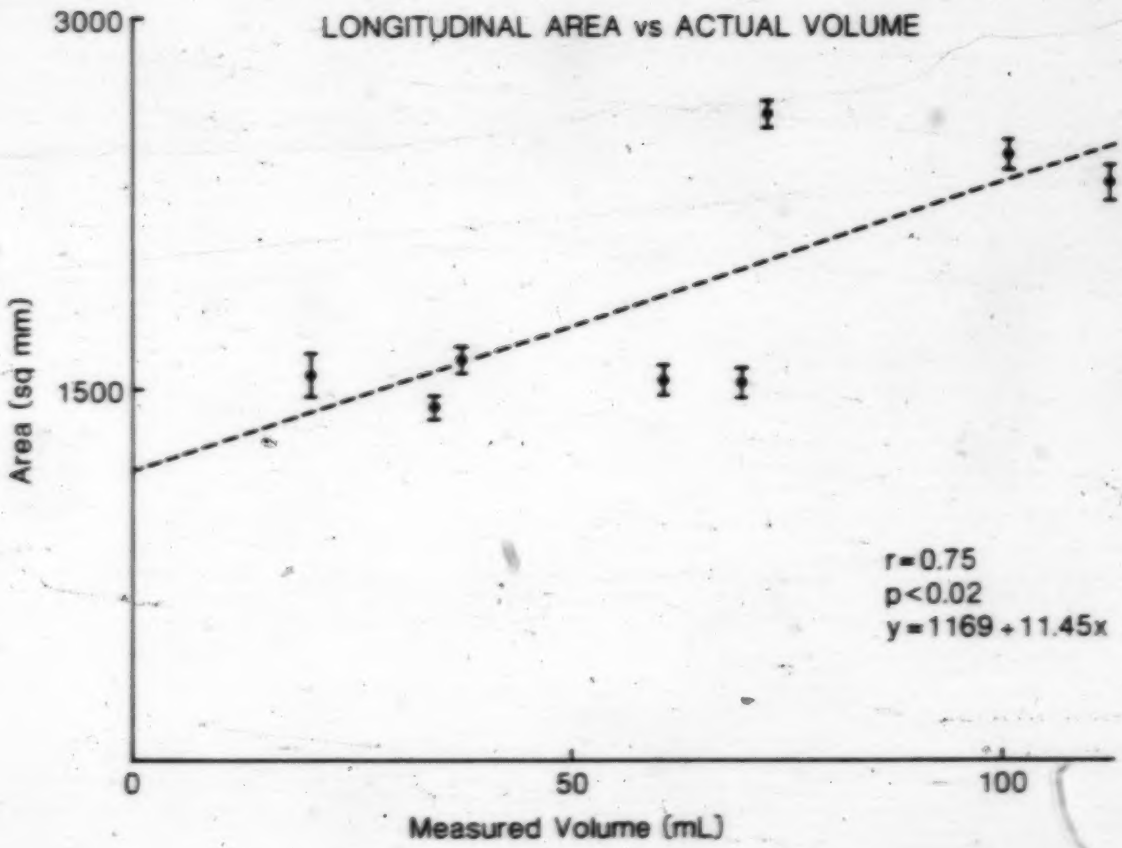
Analysis revealed a significant ($p = 0.003$) reduction in Amax, and a reduction ($p = 0.04$) in b_1, following the course of indomethacin. No other changes were noted.

2. Rectal Indomethacin

Administration of the suppositories caused no discomfort or problem in any of the 6 subjects, but 3 subsequently noted mild tenesmus with the indomethacin suppository only. In no case did this necessitate evacuation prior to completion of the function study.

The gallbladder was imaged at 5 min intervals for 30 min following administration of the suppository and the change in area during this time was compared, placebo vs. test. No significant difference was noted but the gallbladder area tended to increase in both groups.

The function parameters measured in the described manner (Table 9) showed no difference between placebo and test (Table 10).

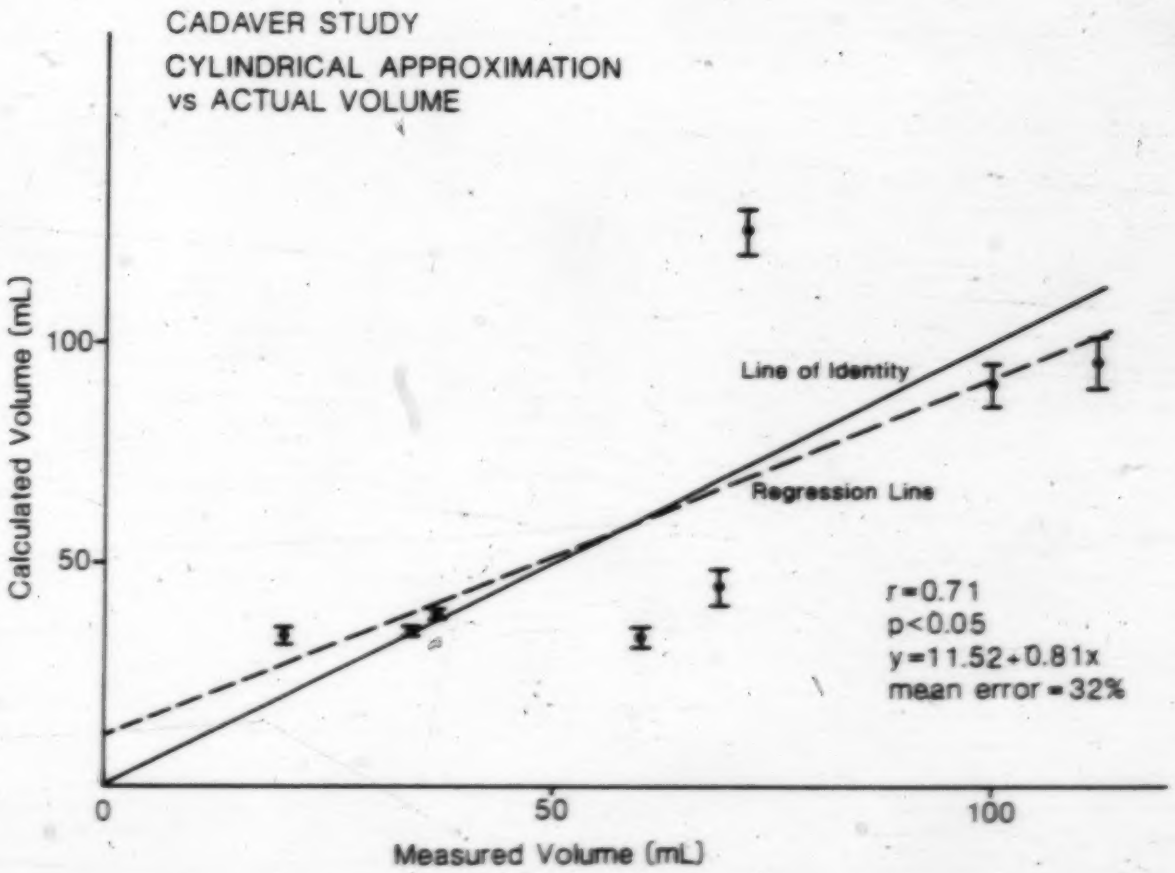


CADAVER STUDY:

THE LONGITUDINAL CROSS-SECTIONAL AREA OF THE GALLBLADDER vs. ACTUAL VOLUME.

THE POINTS REPRESENT THE MEAN ± S.E.M. FOR EACH GALLBLADDER.

FIGURE 7.

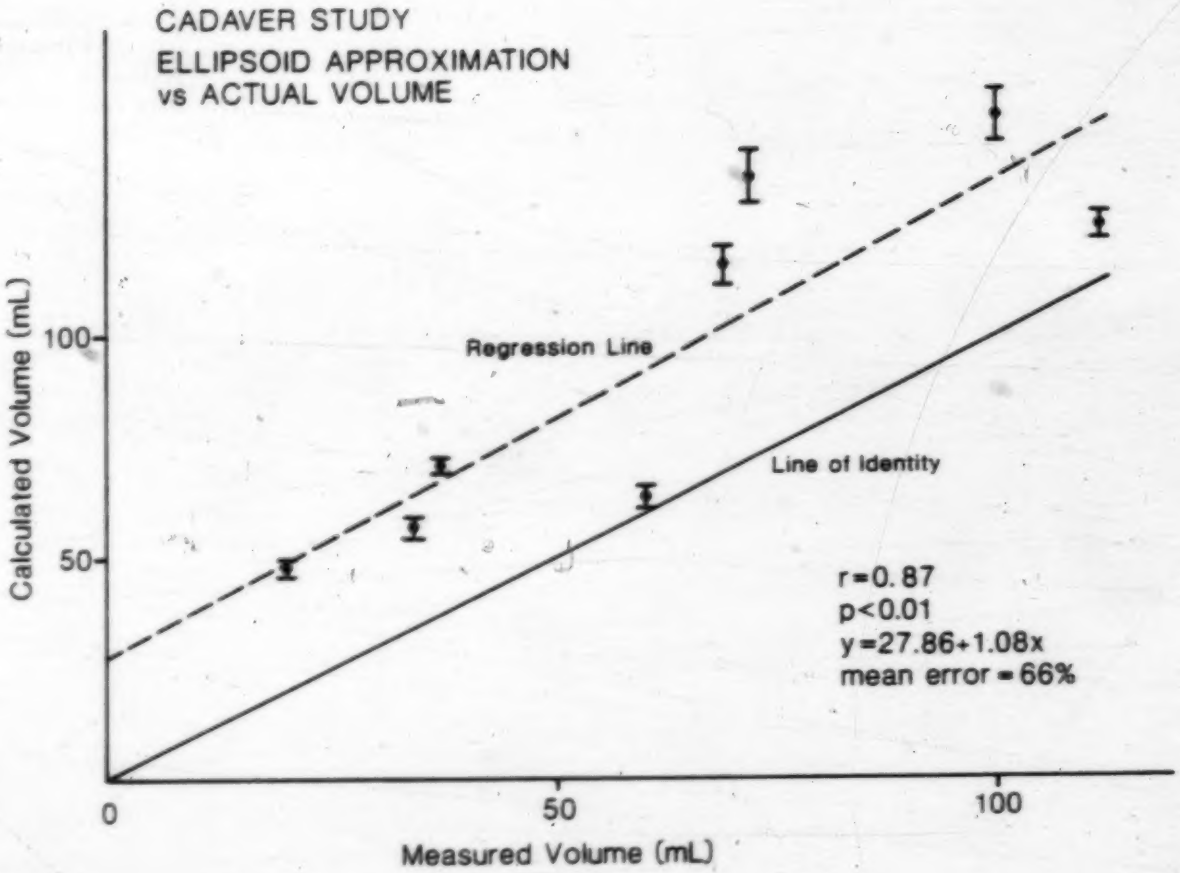


CADAVER STUDY:

THE GALLBLADDER VOLUME CALCULATED BY CYLINDRICAL APPROXIMATION vs.ACTUAL VOLUME.

POINTS REPRESENT MEANS ± S.E.M.

FIGURE 8.

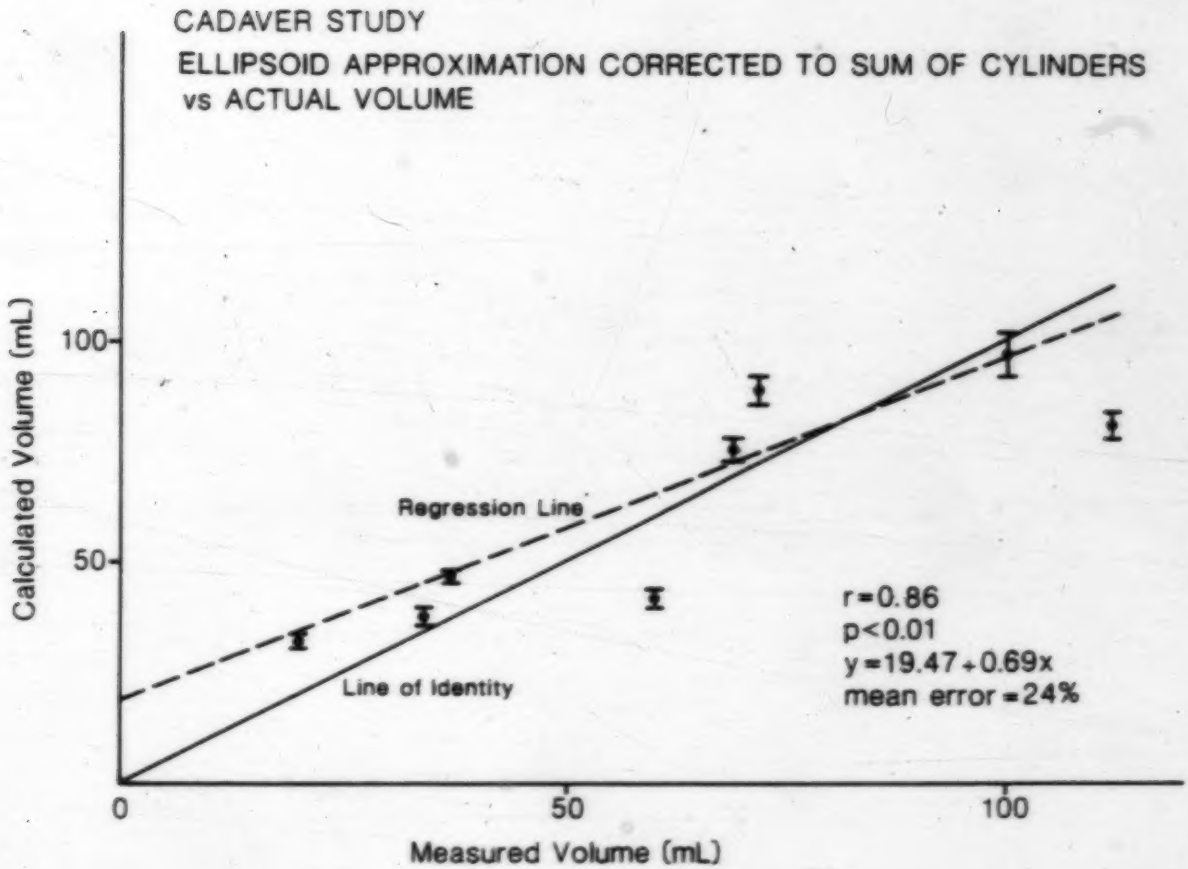


CADAVER STUDY:

THE GALLBLADDER VOLUME CALCULATED BY ELLIPSOID APPROXIMATION vs. ACTUAL VOLUME.

POINTS REPRESENT MEANS ± S.E.M.

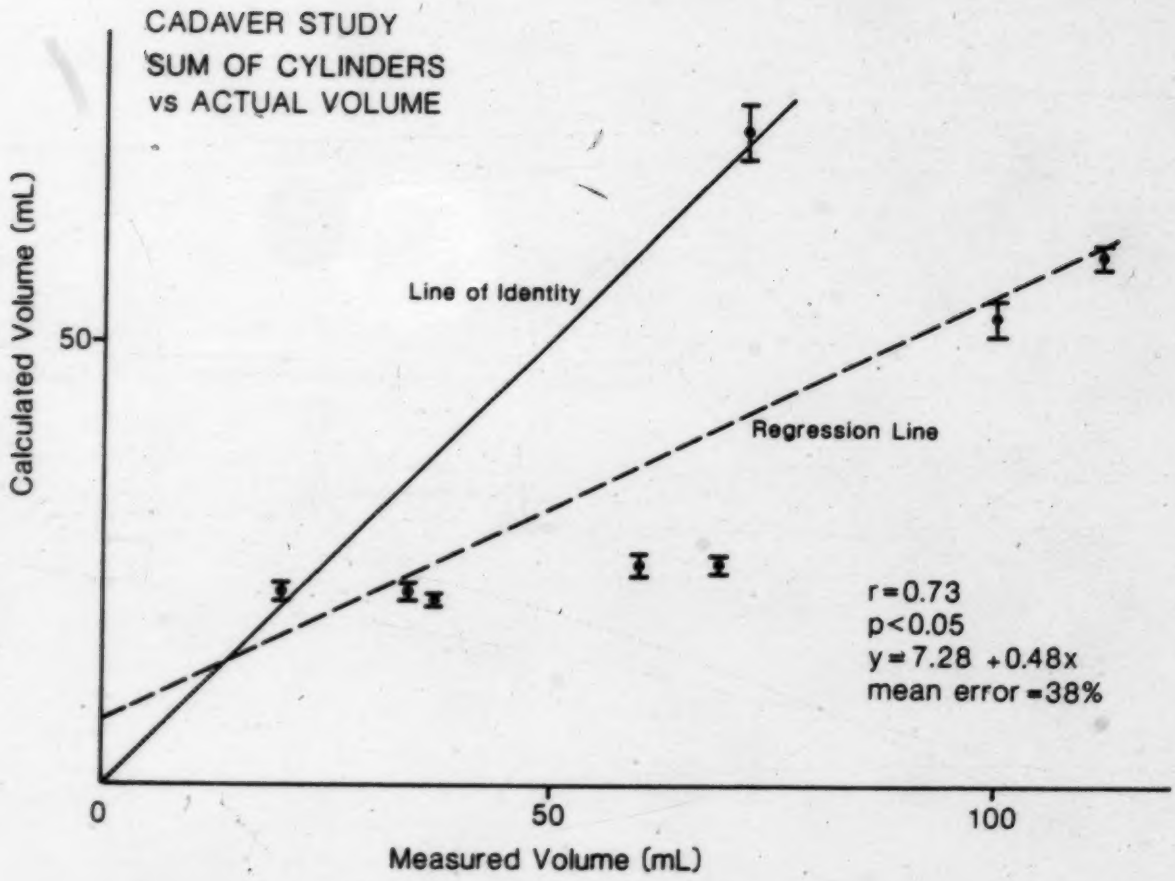
FIGURE 9.



CADAVER STUDY:

THE GALLBLADDER VOLUME CALCULATED BY ELLIPSOID APPROXIMATION CORRECTED TO SUM OF CYLINDERS vs. ACTUAL VOLUME. POINTS REPRESENT MEAN± S.E.M.

FIGURE 10.



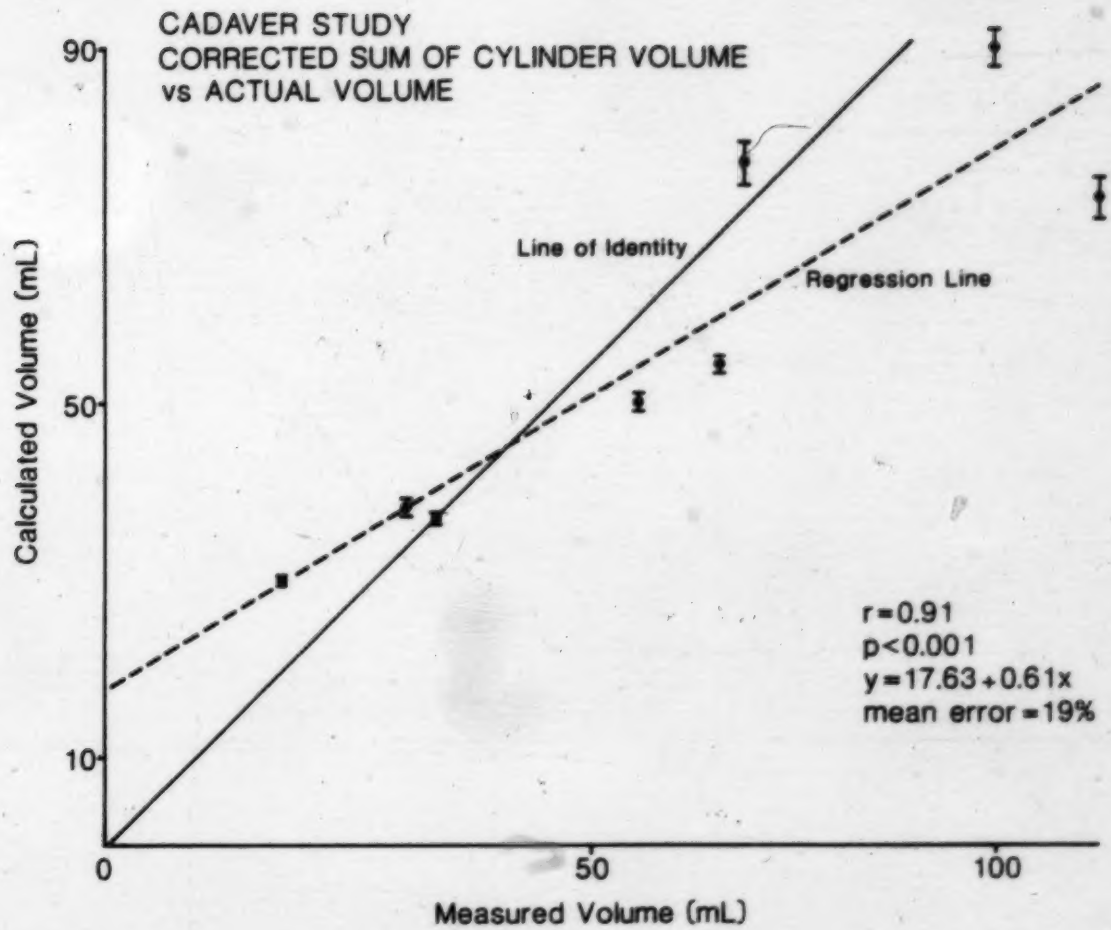
CADAVER STUDY:

GALLBLADDER VOLUME CALCULATED BY SUM OF CYLINDERS

vs. ACTUAL VOLUME.

POINTS REPRESENT MEANS ± S.E.M.

FIGURE 11.

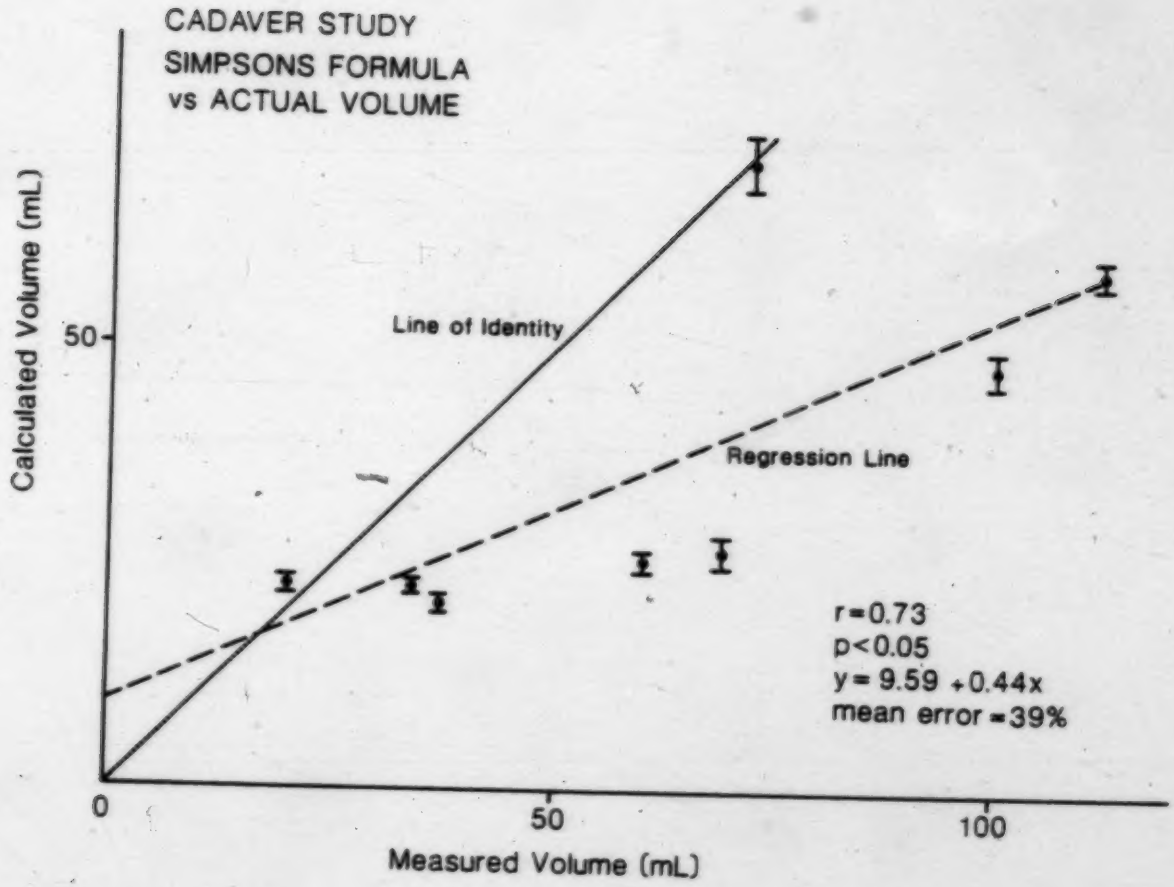


CADAVER STUDY:

GALLBLADDER VOLUME CALCULATED BY CORRECTED SUM OF CYLINDERS vs. ACTUAL VOLUME.

POINTS REPRESENT MEANS ± S.E.M.

FIGURE 12.



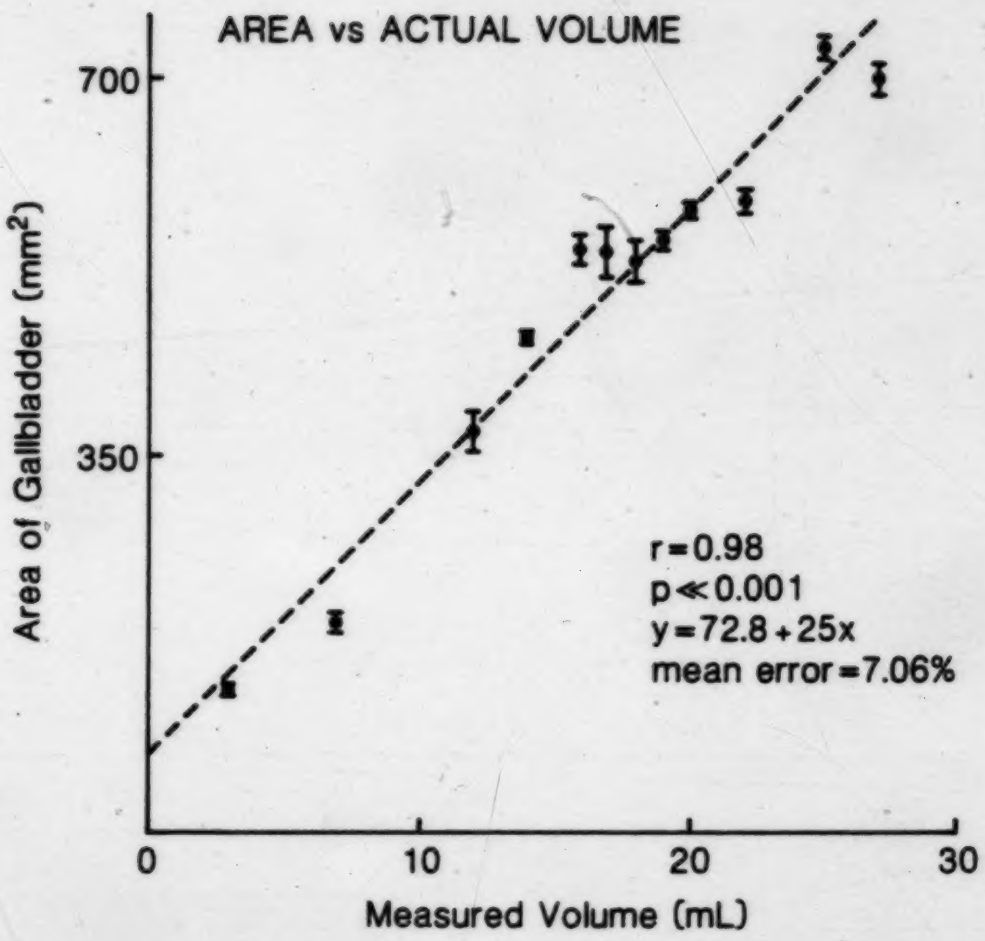
CADAVER STUDY:

GALLBLADDER VOLUME CALCULATED BY SIMPSONS FORMULA

vs. ACTUAL VOLUME.

POINTS REPRESENT MEANS ± S.E.M.

FIGURE 13.

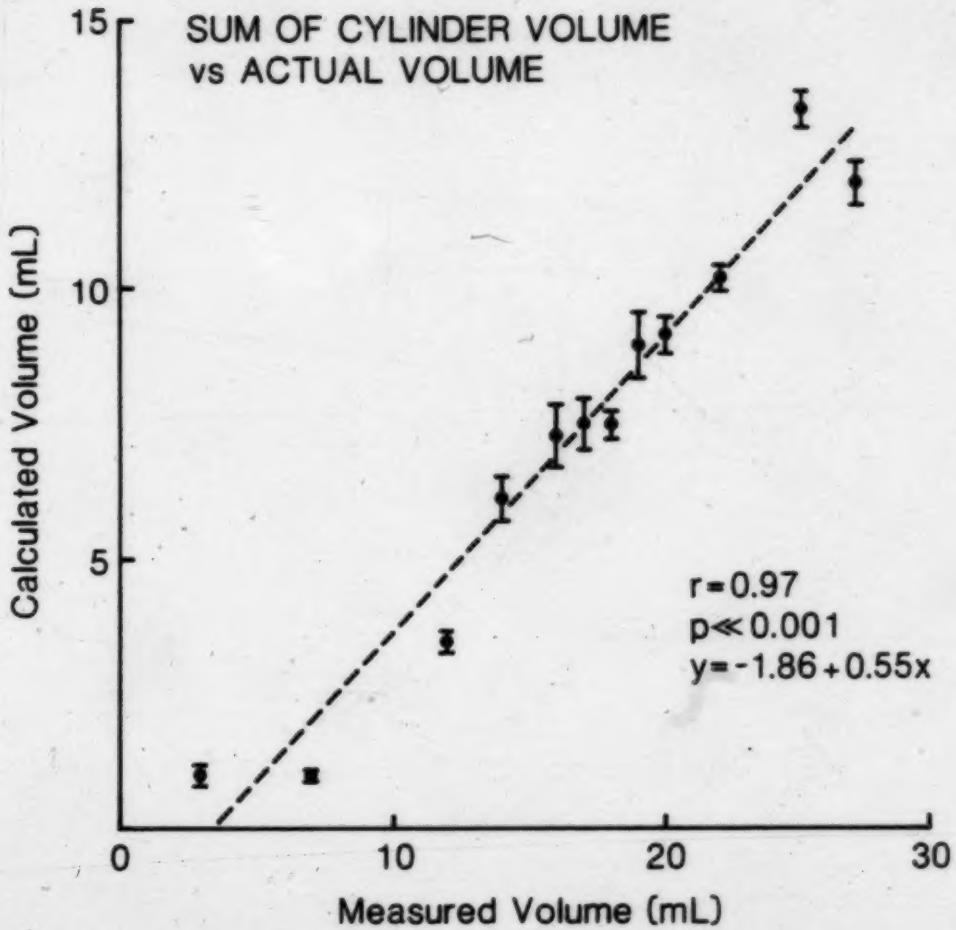


ANIMAL STUDY:

LONGITUDINAL CROSS-SECTIONAL AREA OF THE GALLBLADDER vs. ACTUAL VOLUME.

POINTS REPRESENT MEANS ± S.E.M. OF AREAS

FIGURE 14.



ANIMAL STUDY:

GALLBLADDER VOLUME CALCULATED BY SUM OF CYLINDERS vs. ACTUAL VOLUME.

POINTS REPRESENT MEANS ± S.E.M. OF VOLUMES.

FIGURE 15.

TABLE 1

CADAVER STUDY: GALLBLADDER VOLUMES CALCULATED BY DESCRIBED METHODS

X ± SEM

Measured Volume mls	Area mm^2	Cylindrical Approx. mls	Ellipsoid Approx. mls	Ellipsoid Approx. Corrected To Sum of Cylinders mls	Sum of Cylinders mls	Corrected Sum of Cylinders mls	Simpsons Formula mls
100	2452 ±47	91	150	97	53	90	48
60	1563 ±33	34 ±1	64 ±2	42 ±1	25 ±1	49 ±2	26 ±1
34	1431 ±24	35 ±1	57 ±2	38 ±1	22 ±0.5	38 ±1	23 ±0.5
69	1538 ±47	45 ±3	116 ±4	76 ±2	25 ±1	54 ±2	27 ±1.5
112	2344 ±46	96 ±5	125 ±3	81 ±2	60 ±1	73 ±4	59 ±1
37	1630 ±29	39 ±1	71 ±2	47 ±1	21 ±0.5	37 ±1	21 ±1
20	1549 ±54	34 ±1.5	48 ±1	32 ±1	22 ±0.5	30 ±1	23 ±1
72	2616 ±36	127 ±4	136 ±5	89 ±3	74 ±3	77 ±5	71 ±3
Mean Error%		32	66	24	38	19	39
Coeff. of Correlation (r)	0.75	0.71	0.87	0.86	0.73	0.91	0.73

TABLE 2

Dog Model

Comparison of Longitudinal Cross-sectional Area

of the Gallbladder to its Measured Volume

Volume (ml)	n	Area Mean mm^2	SEM of Areas	CV%	Mean Error %
3	2	1348	42	4	10
7	2	1940	100	7	28
12	4	3723	345	19	0.4
14	2	4570	23	1	7
16	2	5380	260	7	12
17	4	5380	400	15	7
18	2	5302	240	6	1
19	2	5476	92	2	0.3
20	2	5784	23	1	1
22	12	5833	200	12	7
25	6	7200	187	6	3
27	4	6956	200	6	8

Mean % error of area from regression line = 7.06%

TABLE 3

Dog Model

Comparison of Calculated Volume by Sum of Cylinders

Method to Measured Volume of the Gallbladder

Volume (ml)	n	Mean Volume Calculated (ml)	SEM of Volume	Mean % Error From True Volume
3	2	1.01	0.19	66
7	2	1.00	0.03	86
12	2	3.47	0.17	71
14	2	6.17	0.77	56
16	2	7.32	1.18	54
17	2	7.56	0.99	56
18	2	7.48	0.39	58
19	2	9.00	1.10	53
20	2	9.21	0.61	54
22	2	10.26	0.36	53
25	2	13.36	0.47	47
27	2	12.00	0.50	56

Overall Mean error from True Volume = 59.2%

TABLE 4

NORMAL HUMAN GALLBLADDER FUNCTION STUDY PARAMETERS

BASIC STATISTICS

Interindividual n=18	Mean	SEM	CV%
t lag	4.03 min	0.43	46
A rest	1526 mm^2	49.9	14
A max	1774 mm^2	70.6	17
b_o	0.0128 min^{-1}	0.0014	47
b_1	0.0422 min^{-1}	0.0042	42
b_2	0.0058 min^{-1}	0.0009	64
tb_1	15.08 min	1.98	56
$\%E_{30}$	48.6%	2.42	21

Intraindividual n=8	Mean	SEM	CV%
t lag	4.0 min	0.48	36
A max	1550 mm^2	50	10
b_o	0.0137 min^{-1}	0.0014	31
b_1	0.0483 min^{-1}	0.0053	33
b_2	0.0067 min^{-1}	0.0014	62
tb_1	13.7 min	1.26	28
$\%E_{30}$	54.5%	2.6	14

SEM = standard error of the mean

CV% = coefficient of variation percent

TABLE 5

NORMAL GALLBLADDER FUNCTION STUDY

COMPARISON OF PARAMETERS BY LINEAR REGRESSION ANALYSIS

	Intraindividual n = 8	Interindividual n = 18
A max vs b_o	N/S	N/S
A max vs b_1	N/S	N/S
A max vs b_2	N/S	N/S
A max vs tb_1	N/S	N/S
A max vs ΣE_{30}	N/S	N/S
b_1 vs b_2	N/S	N/S
b_1 vs tb_1	N/S	$p < 0.05$ -ve
b_1 vs ΣE_{30}	$p < 0.01$ +ve	$p < 0.001$ +ve
b_2 v tb_1	N/S	N/S
b_o vs ΣE_{30}	N/S	$p < 0.001$ +ve

N/S = not significant

TABLE 6

Oral Indomethacin Study - Gallbladder Function Parameters

Patient Description	b_o min^{-1}	b_1 min^{-1}	b_2 min^{-1}	tb_1 min	$\bar{x}E_{30}$ %	A max mm^2	t lag min
1 control	0.019	0.060	0.004	12.5	64	1648	4
drug	0.013	0.037	0.0065	17	55	1599	2
control	0.0097	0.035	0.001	18	51	1599	4
2 control	0.014	0.025	0.000	35	48	1605	2.5
drug	0.009	0.028	0.002	20	39	1561	2.5
control	0.0097	0.030	0.0066	14	37	1606	4
3 control	0.015	0.04	0.008	12	53	1990	4
drug	0.012	0.043	0.0055	13	50	1531	1.5
control	0.026	0.062	0.009	15	66	1827	5
4 control	0.0043	0.033	0.0026	15	36	2408	4.5
drug	0,012	0.02	0.0067	22	41	2237	3
control	0.0089	0.029	0.0081	8	35	2574	3
5 control	0.016	0.039	0.012	10	45	1555	2
drug	0.013	0.042	0.0013	15	48	1135	7
control	0.011	0.033	0.005	12.5	46	1665	2.5
6 control	0.0099	0.020	0.006	14	38	1703	2
drug	0.0093	0.029	0.0065	16	38	1361	2
7 control	0.006	0.046	0.005	4	33	1636	4
drug	0.010	0.026	0.009	7	39	1423	2.5
8 control	0.015	0.038	0.005	20	54	1531	5
drug	0.017	0.037	0.0112	13	51	1459	5

TABLE 7

Oral Indomethacin Study

Analysis of Variance

To test for homogeneity between the drug and control groups, a one way test of variance was performed.

Analysis for	F		
b_o	0.082	N/S	
b_1	0.475	N/S	F(0.5) 2,18=3.55
A max	1.596	N/S	

There is no difference between the means of the drug and control groups.

To Test for homogeneity between patients, the test was performed the opposite way.

Analysis for	F		
b_o	1.92	N/S	
b_1	2.398	N/S	F(0.5) 7,13=3.57
A max	8.908	significant $p < 0.01$	

For A max therefore, the patients are significantly different. For the other parameters they are not.

TABLE 8

ORAL INDOMETHACIN STUDY

Control vs Indomethacin Treated Groups

Statistical Analysis by Paired t Test, n=9

Parameter	t	p		
b_o	0.29	0.39	N/S	
b_1	2.10	0.04		significant
b_2	-0.31	0.38	N/S	
tb_1	-0.59	0.27	N/S	
t lag	0.50	0.32	N/S	
A max	3.73	0.003		significant
ΣE_{30}	0.35	0.36	N/S	

In each case where two controls existed, the mean was taken and compared to the drug.

TABLE 9

RECTAL INDOMETHACIN STUDY

GALLBLADDER FUNCTION PARAMETERS

Patient	Description	b_0	b_1	b_2	tb_1	$\overline{EF}_{30}$	A max	t lag
1	placebo	0.0123	0.067	0.0049	14	59	1472	4
	drug	0.0114	0.057	0.0087	9	45	1530	5.5
2	placebo	0.012	0.026	0.0049	24	41	2444	3
	drug	0.013	0.033	0.0079	14	42	2413	7
3	placebo	0.0089	0.029	0.0081	8	35	2574	3
	drug	0.0092	0.037	0.0079	4.5	42	2563	2.5
4	placebo	0.012	0.068	0.005	15	60	1475	4
	drug	0.0096	0.0224	0.0078	16	40	1693	3
5	placebo	0.0097	0.030	0.0066	14	37	1606	4
	drug	0.0089	0.025	0.0033	16	40	1719	2.5
6	placebo	0.0095	0.035	0.005	8.5	47	1796	4.5
	drug	0.012	0.0035	0.076	12	50	1628	2.5

TABLE 10

RECTAL INDOMETHACIN STUDY

Placebo V Indomethacin Groups

Statistical Analysis by paired t test; n=6

Parameter	P	
b_o	0.53	N/S
b_1	0.80	N/S
b_2	0.84	N/S
tb_1	0.81	N/S
t lag	0.53	N/S
A max	0.70	N/S
$\%E_{30}$	0.76	N/S

N/S = not significant

DISCUSSION

Validation

The results of the cadaver study show that an isolated estimate of the human gallbladder volume, calculated by any of the methods described is unreliable.

The reasons for this are extrinsic and intrinsic to the method. The extrinsic sources of inaccuracy are the machine (which on calibration was accurate to within 2%), and the hand held transducer. With this real time ultrasonograph it is not possible to encode spatial relationships, and thus transverse and longitudinal scans have to be made visually, both individually and relative to one another. This is difficult even in a "cooperative" cadaver and can be an exagerated source of error in the living subject. The intrinsic sources of error lie in the methods of calculation.

The methods of calculation can be divided into two groups, depending on whether one or two images are used (see pg.41).
There is a difference in the correlation coefficients between the two groups, those methods utilising 2 scans give more consistent results than those using one. Two dimensional methods (single image) must presume symmetry on cross-section, which is patently unwarranted in most gallbladders. Three dimensional methods presume symmetrical semiaxes which is closer to the truth.

Comparison of the mean percentage errors between the two groups does not show the same difference. Volume estimation by the corrected sum of cylinders method[80] (which evolved especially for use with ultrasound) had a mean error of 19%, whereas the other methods were less accurate. The concept of the correction factors to allow for non-

linearity of the gallbladder would seem to account for this. The correction factor allows for the commonplace fact that the echotomographic longitudinal plane is not the same as the geometric longitudinal axis if the gallbladder is curved, (See introduction pg. 22).

It must be remembered in the above, that multiple scans were made in a stationary cadaver, and that if repeated estimations are made in vivo - different errors become manifest. For calculations in vivo, it must be presumed that the compliance of the gallbladder is uniform and, as the volume decreases, the cross-section remains symmetrical and not angulated by the liver bed as often occurs. The presumption is also made that tonic changes will not cause ballooning of parts of the wall. In addition, the relative attitude of the gallbladder may change, and since there is no method of encoding one scan relative to another, different sections may be imaged from the same surface landmark. Finally, in changing from the longitudinal to the transverse plane, there is no guarantee that the gallbladder has not changed in volume or shape.

The results from the dog model, used to simulate these problems, show strong correlations between the actual volume and the longitudinal cross-sectional area ($r=0.98$) or calculated volume ($r=0.97$). The mean percentage error of both these methods from the regression line was small (7%), but the mean error of the calculated volume from the actual volume was large (59%).

This indicates that the possible errors detailed above occur consistently through the range of gallbladder sizes. Unfortunately, for an individual subject, the regression line is unknown but the statement

can be made that incremental changes can be estimated with an accuracy approaching 7%. Furthermore, there is little advantage to be gained in estimating volumes manually, which is time consuming and inaccurate. Rather, the longitudinal cross-sectional area of the gallbladder can be used, the measurement of which, on a digitizing microprocessor, is accurate and rapid.[131]

The methodology validated in this study represents the first objective evaluation to-date in the literature. The limitations of the methods are clearly defined in the human and provides justification for the human gallbladder function test used in the physiological study.

The Human Gallbladder Function Study

Gallbladder function studies lack standardization and the variable results preclude direct comparisons.

1. Protocol for the Test

Females were deliberately excluded owing to conflicting opinion on the effect of the ovulatory cycle on gallbladder motility.[133] The age and size of the male volunteers varied and the effect of these factors is uncertain.[134]

A 12 hour fast for solids and 8 hours for liquids was considered adequate for filling of the gallbladder, but it is possible that MMCs had occurred in some subjects within this time.[135] Boyden noted these changes in 1928,[96] but was unaware of their significance. Thus, the resting volume in a normal subject must be considered to be fluctuating with undetermined effect on subsequent gallbladder emptying.

The liquid test meal empties almost immediately into the duodenum,[96] and this is confirmed by the appearance of fluid in the duodenal bulb on ultrasonography. The time taken for complete gastric

emptying is unknown.

The optimal stimulus for gallbladder contraction is open to debate.[114] Intravenous infusion of CCK is probably the most reproducible of current stimulants, but cannot be considered physiological. Rates of administration vary and some of the drugs are not completely pure.[110] In addition, it is well known that drug adherence and bolus administration can produce pharmacological doses and artifactual results. Intraduodenal infusions suffer similar criticisms.

The test meal used here is both physiological and safe, together with being inexpensive and palatable. Evacuation of the gallbladder was invariably produced and by comparison with others studies, of similar magnitude. The time taken for ingestion was never less than 20 sec or more than 65 sec.

The lag period noted (t lag = 4.0±0.5 min) is the same as quoted by Shaffer for intravenous CCK infusions.[104] . This would indicate that the oral meal releases CCK almost immediately or that other factors potentiate early gallbladder evacuation while the levels of CCK are rising.

2. The Modality

The method by which the gallbladder is imaged has a subtle but profound effect on the results obtained.

Boyden's oral cholecystographic technique utilised one posteroanterior film to estimate gallbladder volume. The silhouette obtained was representative of the cross-sectional area the gallbladder along the geometric longitudinal axis.[96].

According to the law of Laplace (Pierre Simon, Marquis de Laplace, 1749-1827) the pressure in a bubble is proportional to the tension in

its wall and inversely proportional to its radius.

$$P = \frac{2T}{r}$$

Thus, in an irregularly shaped gallbladder, assuming uniform compliance and constant volume, an increase in tension will increase the pressure and/or increase the radius of the eccentric portions of the vesicle, causing it to assume a more spherical shape.

The effect of this on the gallbladder image would be to reduce the apparent volume. This was admitted by Boyden in the above article: "the assertained volume of the flattened organ, when computed as if it were round on cross-section, is larger than its absolute volume".[96] The "two minute pause" could therefore be accounted for by a change in gallbladder tone, and not by any change in volume at all.(See pg. 25).

The associated changes in tone and pressure hypothesised above have been documented in the dog.[136] Following ingestion of a meal, an increase in gallbladder pressure occurs without associated emptying of bile. Approximately 5 mins after the meal, bile is released into the duodenum and the pressure falls. This is supported by Boyden's own observation that bile is not seen in the duodenum for "8 to 14 minutes after the egg yolk"[96]

Ultrasonography, by comparison, utilises a longitudinal echotomogram. As has been described, this may image an area eccentric to the geometric longitudinal axis of the vesicle, and thus yield estimated volumes (or areas) less than those calculated from X-rays. However, since an increase in gallbladder tone will cause the organ to become more spherical, the longitudinal echotomographic plane will more closely approach the geometric longitudinal axis. This will produce an increase in the imaged area, and an apparent increase in the calculated

volume. Thus Amax constitutes the best ultrasonic estimate of the gallbladder size.

The hypothesis is supported by the observation that the form factor increases following the test meal, ($p<0.05$) and the theoretical principle can be shown graphically and quantitated. (Fig.16)

The functional image of the nuclear scan is not complicated by these dimensional problems. Neither an increase in activity nor the two minute pause occur, suggesting again the hypothesis that in the initial "lag phase" the gallbladder volume remains constant. The activity from the gallbladder, as recorded by the gamma camera, depends in part on the distance between the two.[137] Respiration and erection of the gallbladder can alter the distance and cause artifactual changes in the activity registered. Accounting for this, and by using more sophisticated techniques,[110] there is no intermittency of gallbladder emptying, either the "two minute pause" of Boyden,[96] the "humps" of Edholm[95] or the phasic pattern of Englert and Chiu.[138]

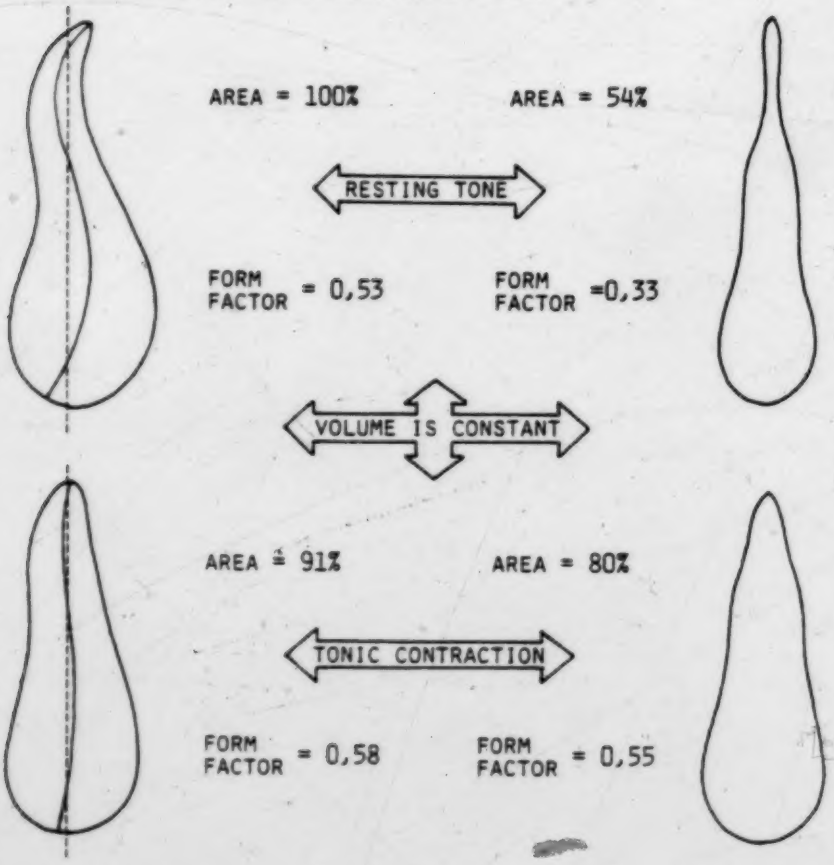
3. Definition of Parameters

The parameters derived from the data depend on the type of analysis applied and the limits imposed.

Parameters such as the A max are fairly well defined, but the rate constants of emptying are critically dependent on the time limits imposed.

The data can be fitted to one of 4 functions; linear, exponential, logarithmic or power curves (see pg. 34). Analysis by the least squares method show that a power curve yields the closest fit (highest coefficient of correlation) but most authors have used the more familiar 1st order exponential function. Since it is probable that more than one

EFFECT OF INCREASING TONE ON GALLBLADDER
LONGITUDINAL CROSS-SECTIONAL AREAS.



Outlines on left represent P.A. projections, as might be obtained with a radiograph.

Outlines on right represent longitudinal ultrasonographs.

The theoretical effects of a change in gallbladder tone on area and on form factor are shown.

FIGURE 16.

variable influences gallbladder emptying, compartmental (1st and 2nd order) exponential analysis seems a logical choice.

This hypothesis is strengthened by the finding that the log data yields two plausible straight lines in all subjects to-date, and statistical analysis shows the rate constants derived are separate and independent variables (Table 5).

Certain parameters are related, for example b_1 and b_o are correlated positively with $\%E_{30}$ as might be expected, and b_1 is correlated negatively with tb_1, which again is not surprising. However, there are no other correlations indicating the independence of these parameters of gallbladder function.

Variation in normal gallbladder function has been demonstrated, and therefore the wide inter-individual range in this study is not unexpected. However, the gallbladder has been documented to behave in a predictable and constant fashion in an individual,[96] and thus the wide intra individual variation shown is notable.

It would be useful to know if this was an effect of the test meal and consequent neural stimulation and CCK release, or due to variations intrinsic to the biliary tree such as the gallbladder muscle, sensitivity to CCK and resistance to outflow of bile. These questions will not be answered without plasma CCK assay and CCK receptor assays.

If the gallbladder muscle obeys Starlings Law,[139] then the degree of filling could influence emptying. Regression analysis of A max against the rate constants fails to show a correlation, indicating that either the human gallbladder muscle does not obey this law, or more likely, other in vivo regulating influences negate this effect. In either case the degree of filling of the gallbladder did not influence

the rate of emptying.

The physiological basis of the rate constants is uncertain. Everson et al.[129] suggested that the rapid and slow phases were due to differential emptying of solids and liquids from the stomach. However, two phases have been shown in this study using a liquid meal, and in retrospect can be inferred from earlier studies using test meals[96] and CCK infusions[126] as stimuli. Gallbladder contraction and reciprocal sphincteric relaxation may be solely responsible,[110] but I would implicate neural stimuli, particularly in the initial phase and possibly resistance at the gallbladder neck and cystic duct in the slow phase. The great variability in the second phase would seem to indicate more than one controlling influence.

Compartmentalising the exponential curve produces a subtle complication. The first rate constant must theoretically be adjusted for the slope of the second order function; an adjustment which has not been made in this study or in the literature.[129] Failure to make this adjustment theoretically invalidates the first order rate constant.

In practice, however, the second order rate constant is much smaller than the first, and may not have a profound effect.

Indomethacin Study

Since gallbladders are removed because of disease, normal human tissue is scarce for in vitro study, and in vivo study is limited by ethical considerations. Ultrasonography therefore provides a unique method of studying the effect of drugs on the normal gallbladder.

All volunteers were considered normal with absence of demonstrable pathology, and the side effects were not considered sufficient to alter the outcome of the test.

The effect of an oral course of indomethacin was to decrease the volume of the gallbladder. This result was unexpected. Prostaglandins produce dose dependent gallbladder contraction, and thus blockade would be predicted to cause dilatation. Although dilatation has not previously been documented, animal studies have shown that indomethacin can reduce the dilatation caused by a high cholesterol diet.[140]

A decrease in gallbladder volume could be due to

1. a decrease in the production of hepatic bile
2. decreased partitioning of bile into the gallbladder due to
    (a) decreased hepatic secretory pressure
    (b) increased resistance in the cystic duct
    (c) decreased compliance or increased tone of the gallbladder.
    (d) decreased resistance of the sphincter of Oddi.
3. increased fluid transport (absorption) and decreased mucus production by the gallbladder.
4. motility changes in the gallbladder, eg., the MMC.

Other than the report of Thornell et al[34] on acutely diseased gallbladders - there are no human studies with which to compare. From animal studies, indomethacin decreases tone[141] and therefore reduces biliary pressure.[140] There may be a decrease in hepatic bile secretion but this has not been documented in humans.

Changes in fluid transport[142] and mucus production[37] are well documented and this must be considered a possible explanation. In contradistinction all available evidence would point to compliance increasing with the drug.[140]

The second observation from this study was the tendency for the

first order rate constant to decrease. Since the physiological controls influencing this phase are not defined, the reason for the change is unknown. However, the volumes in each case are less and this might slow the evacuation although this effect was not confirmed in the physiological study.

The effect could theoretically be accounted for by increased cystic duct resistance or increased viscosity of the bile. Both of these effects would be an acceptable explanation of the first observation as well.

Acute indomethacin administration showed no demonstrable effect on gallbladder function. This would again favour some change in bile partioning or storage as an explanation of the observed effects.

The sphincteric action of the cystic duct is therefore brought into question again,[143] and it would be interesting to know the effects of indomethacin on biliary secretory pressures, sphincter of Oddi resistance, bile osmolality and lithogenic index.

Should there be a change in any of these factors, the possibilities for pharmacological manipulation of the enterohepatic circulation improve. In addition, considering the widespread use of anti-inflammatory agents - may this not be an etiological factor in cholelithiasis?

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APPENDIX 1

Digital Planimetry

The Micro plan II is a digital planimeter designed to collect data from 2-dimensional images. The system consists of an intelligent digitizing tablet with a built in microprocessor and an alphanumeric printer. It calculates perimeter length, enclosed area, form factor, maximum diameter, feret dimensions and center of area for features which are traced on the digitizing tablet. Two populations are stored with totals, means, standard deviations and sums of squares for the measured features.

Area. The area is calculated by an algorithm based on sums and differences of trapezoidal areas within an enclosed area. The area algorithm always connects the starting to the ending points of the segment.

Form Factor. Form factor is a dimensionless ratio of enclosed area to the perimeter, normalised so that for a circle, Form Factor = 1 and for a line it is 0. The relationship is:

$$\text{form factor} = 4\ (\text{pi})\ \frac{A}{p^2}$$

Form factor is often used to assess the roundness of objects.

APPENDIX 2

Analysis of the Gallbladder Function Study and Derivation of Parameters

The ultrasonographic longitudinal area was measured in each case and plotted as the logarithm against time (Fig. 6). By inspection, two straight lines were drawn to approximate the log linear first and second phases of evacuation. The data for the first and second order functions were fitted to an exponential curve by the least squares method using a Hewlett Packard (H.P. 97) programmable calculator. The limits are determined by inspection from the graph.

b_o the overall rate constant; regressed from the first obvious decrease in area for 60 min following the test meal.

b_1 the rate constant of the initial phase of evacuation regressed from the first obvious decrease in area to the intersection of the two log linear phases drawn on the graph.

b_2 the rate constant of the second phase regressed from the point of intersection of the two log linear phases on the graph to 60 mins or when the data deviates from log linearity.

The remainder of the parameters were determined by direct inspection of the data and graph.

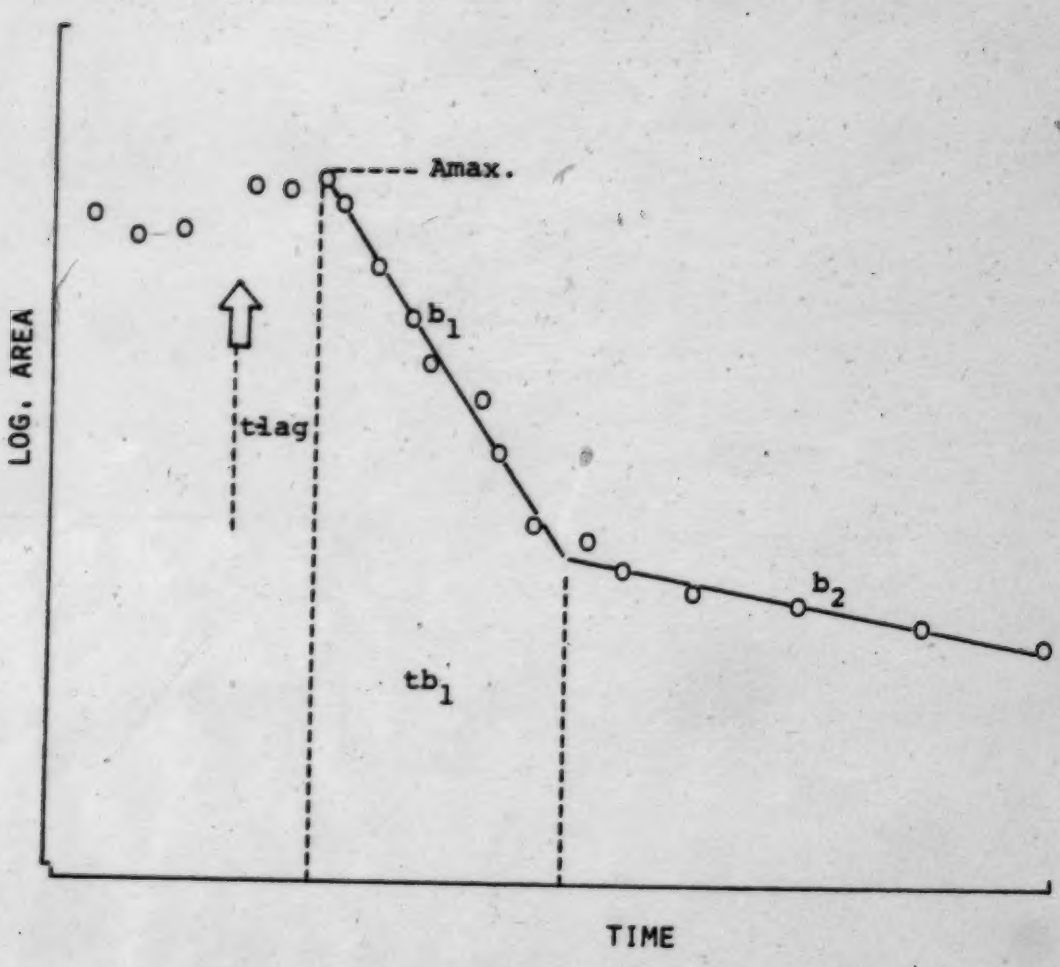
A max the maximum area measured after the test meal.

A rest the mean area recorded in the 10 min period before the test meal.

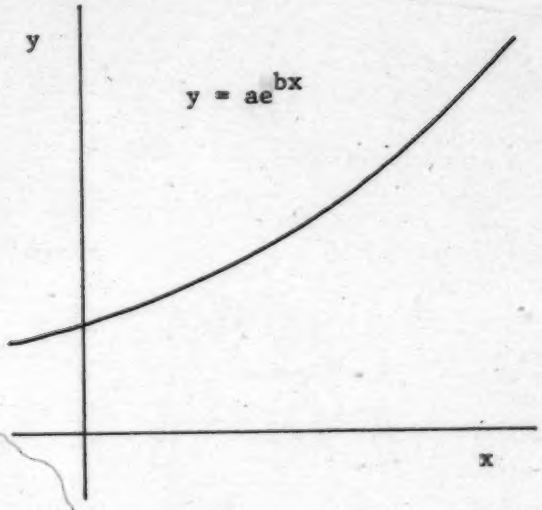
t lag the time period between the start of the ingestion of the meal and the onset of gallbladder evacuation.

tb_1 the time period of the rapid phase of emptying.

ΣE_{30} the percentage change in area during the 30 min period from the ingestion of the test meal.



EXPONENTIAL CURVE FIT.



$$b = \frac{\Sigma x_i \ln y_i - \frac{1}{n}(\Sigma x_i)(\Sigma \ln y_i)}{\Sigma x_i^2 - \frac{1}{n}(\Sigma x_i)^2}$$